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Evaluation of an indwelling bolus equipped with a triaxial accelerometer for the characterisation of the diurnal pattern of reticuloruminal contractions

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Author's Declaration

I declare that, except where explicit reference is made to other people's contributions, this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Name: Giovanni Capuzzello

Signature:

Abbreviations

AUSCICI auscultation inter-contraction interval

BCD bolus contraction duration

BICI bolus inter-contraction interval

BRT bolus rumination time

CD contraction duration

CET collar eating time

CRT collar rumination time

DA displaced abomasum

DMI dry matter intake

ICI inter-contraction interval

RRCR reticuloruminal contraction rate

RP retained placenta

SARA subacute ruminal acidosis

USICI ultrasound inter-contraction interval

1 Introduction

1.1 Anatomy of the reticulorumen

The ruminant's gastrointestinal tract is a marvel of evolution, which allows ruminants to efficiently break down and ferment fibrous plant particles. The gastrointestinal tract includes three forestomachs (reticulum, rumen and omasum) and one glandular stomach (abomasum). These four compartments, collectively, occupy about 80% of the abdominal cavity, with the reticulum being the smallest and the rumen being the largest (containing up to 150 Litres of ingesta).

1.1.1 The reticulorumen

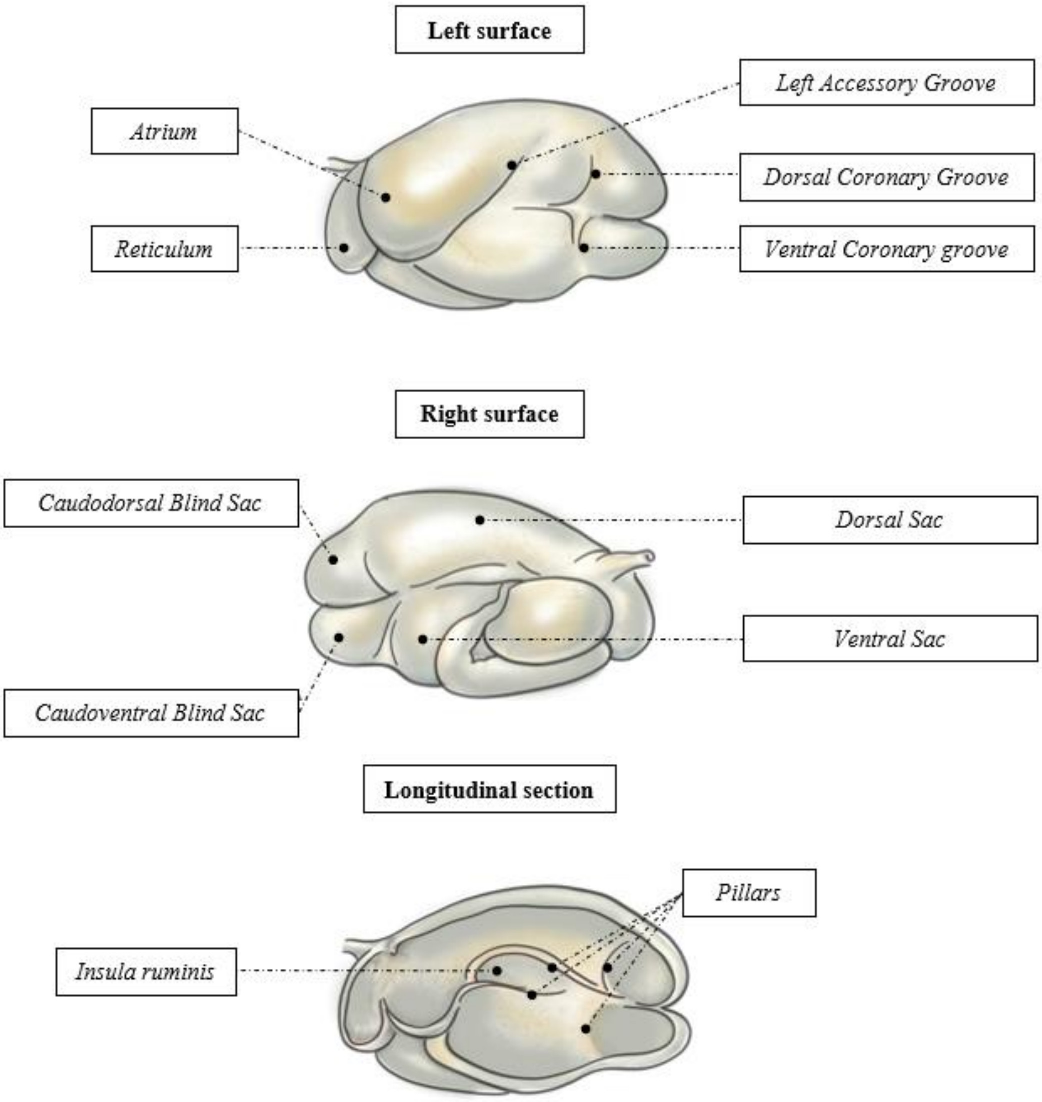
The first two forestomachs, the reticulum and the rumen, can be considered together as a unique structure called the reticulorumen.

The reticulum is the most cranial of the forestomachs. Externally, it has two surfaces, the diaphragmatic and the visceral. On the diaphragmatic, the reticulum is in contact with the diaphragm only. On the visceral side, the reticulum is in contact caudally and dorsally with the rumen; ventrally, it is separated from the rumen by the reticulorumen groove (Fig.1.1). Internally, the mucosa has crests 1 cm high, forming honeycomb-like cells (Barone and Bortolami, 2014). Histologically, the internal surface of the reticulum is covered by four layers (Liebich and Kupliec, 2019). The first layer consists of a stratified keratinised squamous epithelium cell. This layer is morphologically distinguished by reticular crests (*cristae reticule)* that constitute four to six layers of reticular cells *(cellular reticule).* On the sides of the crests, conical papillae are observed; a lamina muscularis is present in the superior part of the crests. The second layer is the submucosa. The third layer is the tunica muscularis, which comprises circular and longitudinal muscle layers. The most external layer is the tunica serosa (Liebich and Kupliec, 2019)**.**

The **rumen** occupies the left and medio-ventral part of the abdominal cavity. Longitudinally, it is divided into the dorsal and the ventral sac, which both end in a cul-de-sac. The cranial part, called "atrium", is connected with the oesophagus and the reticulum (Barone and Bortolami, 2014). Externally, it has two surfaces, the parietal and the visceral surfaces. The parietal one is in contact with the diaphragm, spleen and abdominal wall, whereas the visceral one is in contact with the intestine, liver, omasum and abomasum (Barone and Bortolami, 2014). Both surfaces are shaped by grooves, namely the ventral and the dorsal coronary grooves (Figure 1-1). Two curvatures can be observed: the dorsal and the ventral curvatures; the dorsal curvature lies against the diaphragm and roof of the abdominal cavity; on the other hand, the ventral curvature lies against the abdominal floor (Barone and Bortolami, 2014).

Internally, rumen papillae (*papillae ruminis*) can be observed on the inner surface; they vary considerably in density, form and distribution. Other structures macroscopically visible are the pillars; the cranial and caudal pillars are the most important ones, and they are divided into smaller branches (Figure 1-1). Different pillars are involved during different phases in the contraction of rumen.

Histologically, from the inner to the outer, the rumen is characterised by a non-glandular mucosa, submucosa, tunica muscularis, and tunica serosa. The presence of the papillae characterises the inner layer. Collagen fibres form the submucosa. The tunica muscularis is characterised by the presence of smooth muscle, which includes a circular (inner) and longitudinal layer (external). The outermost layer is the tunica serosa; a tunica adventitia is present between the rumen and spleen.



Left surface

Right surface

Longitudinal section

Figure 1-1 Anatomy of the internal and external conformation of the reticulorumen (created by Marta Grunt based on Marchionatti 2022 Anatomy of the forestomachs; In Orsini, J.A., Grenager, N.S. & DeLahunta, A. 2022, Comparative veterinary anatomy: a clinical approach, Elsevier, Academic Press, an Imprint of Elsevier; Kidlington, Oxford, United Kingdom; page 1059).

1.1.2 Innervation and blood supply of reticulorumen

The innervation of the reticulorumen is complex, with the origin in the gastric centres of the medulla oblongata. The right and left vagus nerves are connected to the effector organs (forestomachs) and divided into ventral and dorsal branches (after the carotid sheath). The dorsal branches (right and left) are re-united at the level of the 9th thoracic vertebrae, whereas the ventral branches pass caudally to tracheal bifurcation originating a ventral and dorsal vagal trunk (Fig 1.2). The ventral trunk has several branches which transfer the impulse to a portion of the reticulum, the abomasum, and the ventral parietal surface of the omasum. The dorsal branch has a more prominent function over the rumen. Some studies demonstrated that ventral vagotomy partially affects reticuloruminal motility, whereas dorsal vagotomy can interrupt the normal functionality of the organ (Constable *et al.*, 2017). Blood supply is given mainly by the celiac artery; two main branches, the left and the right ruminal arteries, originate from the celiac artery and end in the ruminal arteriole on the mucosal surface. Similarly, veins originate from the mucosal surface and develop into bigger blood vessels on the rumen surface, which terminate in the portal vein (Fig.1.3).

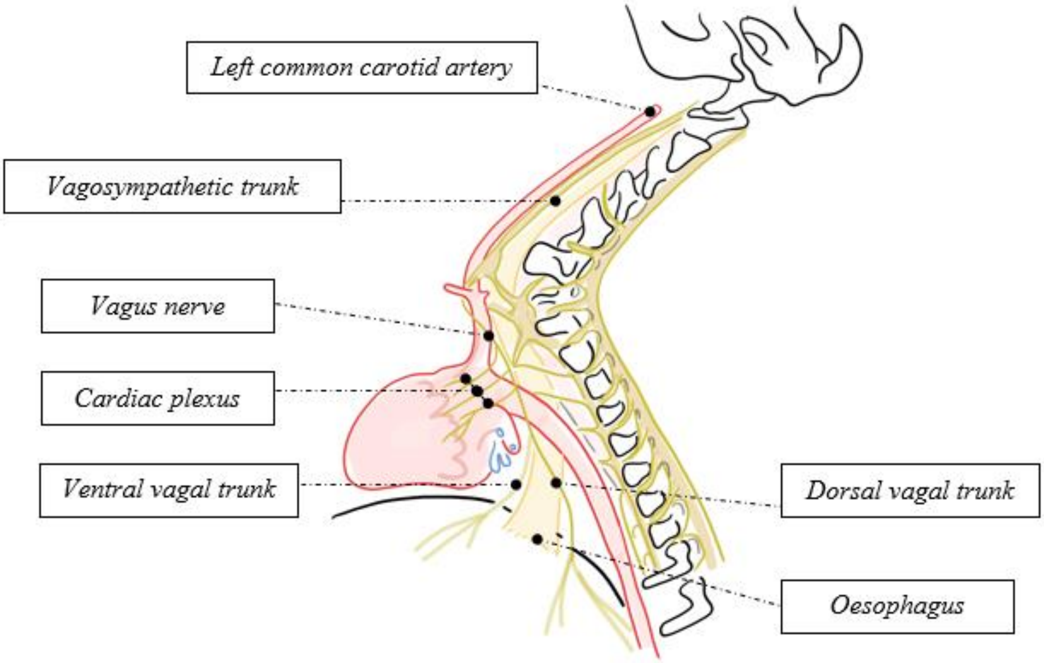


Figure 1-2 Schematic representation of the vagal trunk pathway (created by Marta Grunt based on Spinal cord and autonomic nervous system. In; Budras, K. 2003, Bovine anatomy: an illustrated text, Schlütersche, Hannover. Page 73).

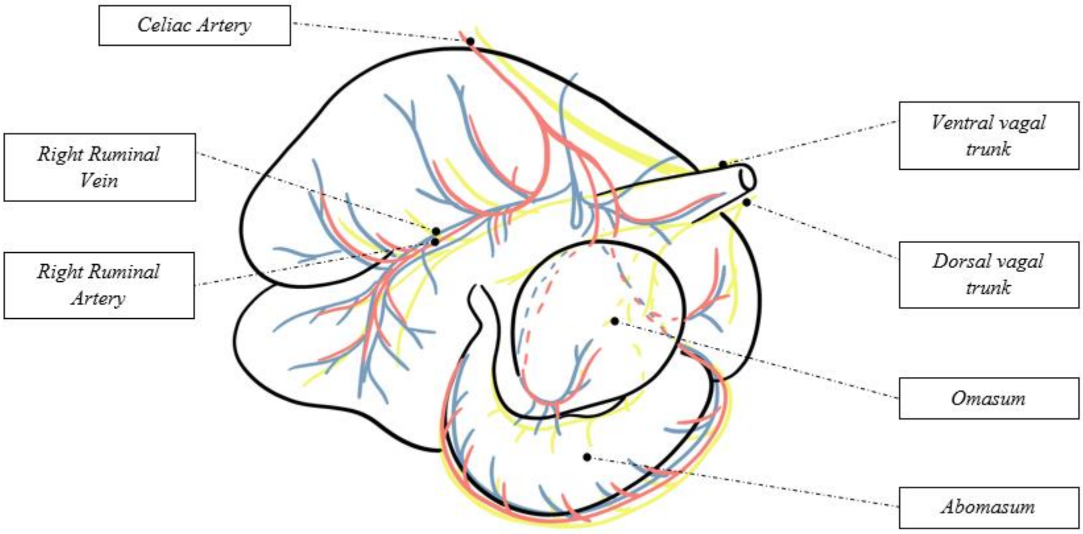


Figure 1-3 Blood supply and innervation of the reticulorumen, right surface view (created by Marta Grunt based on Blood supply and innervation of the forestomachs: lymph node and omenta. In; Budras, K. 2003, Bovine anatomy: an illustrated text, Schlütersche, Hannover. Page 73).

1.2 Physiology of forestomach motility

Four different patterns of contractions can be recognised (Sjaastad *et al.*, 2016):

1- Primary contractions (or mixing cycle)
2- Secondary contractions (or eructation cycle)
3- Rumination
4- Oesophageal groove closure

1.2.1 Primary contractions

Primary contractions commence with a reticular biphasic contraction, followed by a contraction of different parts of the rumen in a craniocaudal order. A more detailed sequence of the primary contractions and their characteristics is described below.

Sequence of contractions

During the biphasic reticular contraction, the first contraction is characterised by an intense narrowing of the reticulum, during which the organ contracts to half its internal volume (Sellers and Stevens, 1966). The second contraction of the reticulum is the most powerful of the two and the lumen of the reticulum is almost completely obliterated (Sellers and Stevens, 1966). After the second contraction, the wave of the contraction passes to the rumen. Subsequently, the reticulum quickly relaxes and remains in this state until the next series of contractions begin. Between the first and the second reticular contraction, a short period of muscular relaxation is observed (Sellers and Stevens, 1966).

Characteristic of primary reticular contractions (Duration, amplitude, speed and number of contractions)

Braun and Rauch (2008) evaluated, by ultrasonography, the characteristics of the first and second contractions, measuring their duration (seconds), amplitude (centimetres) and speed (centimetres/seconds). The first contraction duration varied between 2.4 and 2.9 seconds, amplitude between 8.3 and 8.7 centimetres, and speed between 6.5 and 7.1 cm/s. The second contraction duration varied between 4.0 and 4.3 seconds, with an amplitude between 9.9 and 11.2 centimetres (Braun and Rauch, 2008). The mean number of reticular contractions at rest in 9 minutes was 10.9. Similarly, the mean number of reticular contractions while eating and during rumination was 13.9 and 9.7 in the same period (Braun and Rauch, 2008). The study evaluated the reticular contractions under a stressful situation such as a trimming session; in this case, the reticular contractions mean was 9.3 in 9 minutes. Single contractions occur very rarely. Biphasic contractions are the most common pattern, although animals can have a triphasic contraction. More rarely, four phasic contractions are described (Braun and Schweizer, 2015).

1.2.2 Secondary contractions

In this part of the cycle, the dorsal and ventral sacs contract to move the ingesta, whereas the reticulum and atrium do not contract in this phase. Secondary contractions require 30 seconds to be completed in cattle and are usually, although not always, associated with eructation. Ruckebusch and Tomov in 1973 demonstrated that secondary contractions originated in the ventral sac, independently or immediately after the primary contractions. The wave of contraction was seen to pass circularly to the dorsal blind sac, dorsal sac and ventral sac and back to the ventral blind, with eructation occurring at the end of the contraction of the dorsal sac; the time required to complete a cycle wave was related to the contraction strength of the ventral blind sac. It is essential to mention that the events result in a gradual wave of contraction followed by a wave of relaxation, with some sacs dilating while others contract. For example, the ventral sacs and reticulum dilate when the dorsal sac contracts.

1.2.3 Rumination

Rumination, commonly referred to as cud chewing, is a distinctive behaviour observed in ruminants. This process involves the regurgitation of food from the rumen back into the mouth, where it is chewed again and then swallowed for further digestion (Beauchemin, 2018). It occurs when an extra-reticular contraction takes place before the biphasic contractions. Synchronously with the extra-reticular contraction, the cardia relaxes; this relaxation, in turn, creates a negative pressure within the thoracic cavity. The negative pressure facilitates the movement of the fermented food contents (ingesta) back up through the oesophagus and into the mouth, where it can be chewed again. This process allows ruminants to break forage further and expose new parts of the plant for bacterial fermentation. The sequence of reticuloruminal contractions is synthesised, in Figure 1-4. In this manuscript, the reticuloruminal contraction rate (RRCR) refers to the biphasic contractions (primary cycle) and the extra-reticular contractions (rumination).

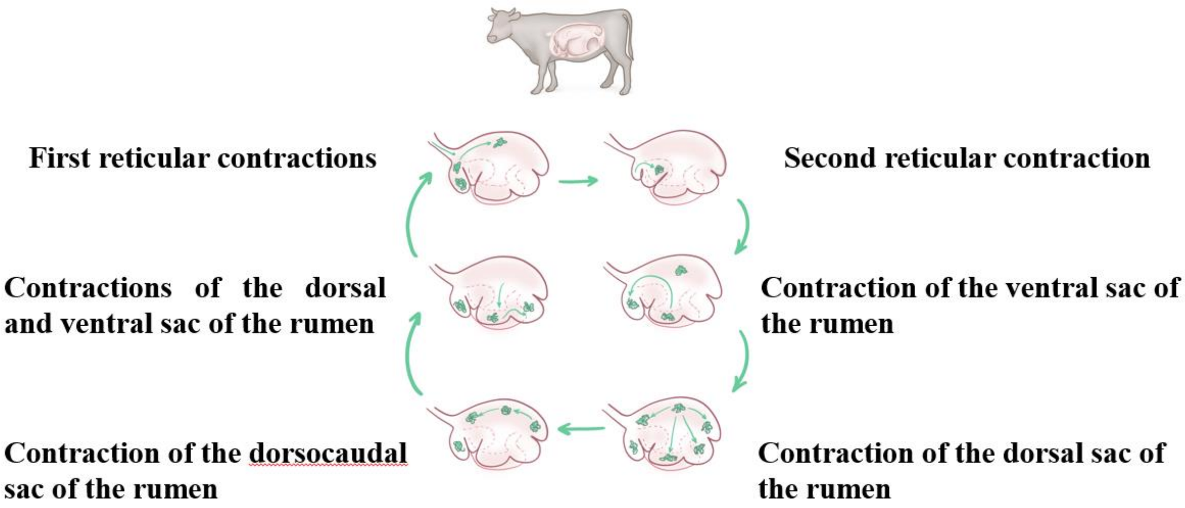


Figure 1-4 Graphic representation of reticuloruminal contractions (created by Marta Grunt based on Marchionatti 2022 Pattern of ruminant forestomachs contraction; In Orsini, J.A., Grenager, N.S. & DeLahunta, A. 2022, Comparative veterinary anatomy: a clinical approach, Elsevier, Academic Press, an Imprint of Elsevier; Kidlington, Oxford, United Kingdom; page 1061).

1.3 Modulation of reticuloruminal motility

1.3.1 Physiological factors affecting reticuloruminal motility

Effect of eating

When eating, the reticuloruminal motility increases to improve the mixing of the ingesta; in particular, the number and the strength of contractions are increased (Beauchemin, 2018).

Effect of fasting and feeding

On the other hand, post-feeding, the amplitude and frequency of the contractions increase (Beauchemin, 2018). Time spent ruminating is reported to peak about 4 hours after feeding (Schirmann *et al.*, 2012).

Effect of fasting

According to studies conducted on fasted sheep, frequencies and durations of reticuloruminal contractions are lower. In addition, a lower rate of secondary contractions has been reported. A compensatory behaviour is observed when feed access is restricted to cows, with cows increasing the time spent ruminating (Dulphy *et al.*, 1979; Campling and Morgan, 1981).

Effect of diet

The consequence of the different diets on reticuloruminal motility is well described in the literature and is affected by the chemical and physical characteristics of the diet (Beauchemin, 2018). Generally speaking, when cattle are fed a high-forage diet, the rumination time (minutes/ kg of DMI) is greater than when cows are fed with concentrate diets (Beauchemin, 2018); however, with long fibre diets, variation from 75 to 180 minutes/day is observed according to what type of forage is fed to cows (Beauchemin, 2018). Regarding the type of contractions, cows fed long fibre (≥ 19 mm) tend to lower the amplitude and reduce the rate of contractions (Tafaj *et al.*, 2007). In a study by Hamilton *et al.* (2019), the amplitude and the inter-contractions period were assessed by a triaxial accelerometer bolus in the reticulum when two diets were fed: high starch and high sugar. In particular, the high starch diet increased the amplitude of the reticuloruminal contraction. A 2016 meta-analysis showed that decreasing the mean particle size from a mean of 10 to 6.7 mm decreases the rumination time by 28 minutes/day (Nasrollahi *et al.*, 2016)**.** Various authors have tried to predict rumination time according to different components in the diet. Still, accuracy has been low, with a root mean squared error (RMSE) ranging between 4.2 and 17.3% (Beauchemin, 2018).

Effect of resting, sleeping and lying

The relationship between resting, lying and sleeping and reticuloruminal motility is ambiguous. Although rumination occurs mostly at night when cows lie down, studies disagree on whether the cows can be asleep or are always awake when ruminating (Tucker *et al.*, 2021). Some studies report that reticuloruminal contractions decreased or stopped when resting and sleeping; on the other hand, the causal relationship between reticuloruminal motility appears to be unclear in other studies (Klemm, 1966; Bell and Itabisashi, 1973; Ruckebusch, 1974).

Effect of water intake

Research on the effect of water intake on reticuloruminal motility is limited (Williams *et al.*, 2020); however, the assumption is that the reticulorumen requires sufficient fluid for the contractions to proceed (Gordon, 1965). Drinking causes an increase in the rate of contractions; conversely, when cows are water-deprived, reticuloruminal motility decreases, although the extent depends on the number of hours/days of water deprivation (Gordon, 1965; Beauchemin, 2018).

Animal individual variability

Individual variability is reported in research mainly for rumination time. The coefficient of variation among cattle for the rumination time (min/h) is reported to be between 16 % and 48 %, depending on the studies considered (Dado and Allen, 1993; Byskov *et al.*, 2015).

1.3.2 Pathological factors affecting reticuloruminal motility

The regulation of reticuloruminal contractions involves several control factors: low-threshold reticular tension and high-threshold tension receptors, abomasal tension receptors, aspecific painful stimuli, changes in the volume and consistency of rumen content, hydration state, electrolytes and acid-base balance (Table 1.1). Below is a brief description of the pathophysiology of factors affecting reticuloruminal motility.

Factors decreasing reticuloruminal motility

Pyrexia

Pyrogenic substances directly affect the gastric centres in the hypothalamus, decreasing reticuloruminal motility; hypomotility and atony are observed in diseases caused by increased body temperature. In a study conducted by Payudal et al., 210 multiparous Holstein cows were monitored at the University of Florida Dairy Unit using rumination loggers. Fever, identified as a rectal temperature above 39.5°C, was among the health disorders assessed, affecting the rumination patterns significantly. Cows with fever demonstrated a noticeable decrease in rumination time.

Endotoxemia

It is caused by some diseases in which toxins are released either by endogenous (i.e., self-produced by the body) or exogenous pathways (i.e., bacteria). Two main pathophysiological mechanisms have been identified; one is prostaglandin-associated, and another one is temperature-associated. Endotoxemia can cause a marked reduction of reticuloruminal motility; other clinical signs include fever and anorexia, which can lead to rumen atony. In a study conducted in 1992 by Eades, six adult, nonlactating, nonpregnant Holstein cows were subjected to varying doses of *Escherichia coli* endotoxin to investigate its impact on reticulorumen motility. The highest dose of 100 ng/kg notably reduced the frequency of reticulorumen contractions and induced fever, indicating reticulorumen stasis linked to enhanced thromboxane and prostacyclin synthesis.

Pain

It can directly affect the gastric centres; the painful stimuli created by the distension of organs in the abdominal cavity can be caused by the release of catecholamine. In addition, splanchnic motor nerves can be stimulated by pain, causing an inhibition of the reticuloruminal motility (Constable *et al.*, 2017). In a study conducted by Rial et al., 871 Holstein cows were enrolled to assess the effects of metritis and clinical mastitis on rumination, physical activity, and lying behaviour using ear-attached sensors. The study included cows with severe metritis and severe clinical mastitis, both recognised as painful conditions. Specifically, 25 cows developed severe metritis, and 15 cows developed severe clinical mastitis, characterised by systemic signs of disease, including pyrexia. Both conditions resulted in significant reductions in rumination time and physical activity, and increases in lying time, reflecting substantial discomfort and behavioural changes in the affected cows.

Distension of forestomach

Tension receptors located in the reticulum and rumen respond to mechanical distension of these organs, creating an inhibitory effect on reticuloruminal motility; this is typically seen in diseases such as reticulorumen traumatic pericarditis or torsions of the intestine (Foster, 2017). In the study by Ismail et al., 31 adult dairy cattle suffering from recurrent rumen tympany were investigated through exploratory laparotomy and rumenotomy. These cattle were categorised based on surgical findings into three groups, with variations in the presence of metallic and non-metallic foreign bodies and perireticular adhesions. Among these, 11 animals (35%) exhibited mild tympany without apparent respiratory distress, while 20 animals (65%) experienced moderate to severe tympany that included varying degrees of respiratory distress. Rumen motility was reduced across all groups, indicating that both the presence of foreign bodies and the severity of tympany adversely affected ruminal motility.

Ruminal volatile fatty acid

Epithelial receptors can detect an increased concentration of volatile fatty acids, which can stimulate a tonic inhibitory input to the gastric centres; this is typically observed when ruminants develop lactic acidosis (Gregory, 1987). In the study by Gregory (1987), sheep were used to investigate the impact of volatile fatty acids (VFAs) on ruminal motility. The sheep were subjected to ruminal, abomasal, and duodenal infusions of VFAs (acetic, propionic, butyric) to evaluate changes in motility patterns, which were recorded via electromyography. The results demonstrated that VFAs significantly inhibited ruminal motility, particularly by reducing the amplitude of the reticulo-ruminal contractions. This inhibition was dose-dependent, with higher concentrations of VFAs leading to more pronounced effects. The study indicates that VFAs can substantially affect the motility of the rumen, suggesting a direct impact of these acids on ruminal muscle activity.

Abomasal diseases

In the abomasum, tension receptors detect excessive distension of the organ, causing inhibition of abomasal motility and reducing the flow rate of ingesta into it (Foster, 2017). In a study by Wittek et al. (2009), the focus was on 30 lactating dairy cows diagnosed with left displaced abomasum (LDA). These cows underwent surgical correction to address their abomasal displacement. The study primarily aimed to evaluate the impact of LDA on abomasal emptying rate, a critical aspect of digestive function. The findings indicated that cows suffering from LDA experienced a significant decrease in abomasal emptying rate. This condition of delayed abomasal emptying reflects the broader effects of LDA on reticuloruminal motility, where the normal function and coordination of the stomach sections are disrupted due to organ displacement.

Effect of depressant drugs

Some drugs have a well-known effect on reticuloruminal motility; in particular xylazine, by activating alpha 2-adrenoceptors and reducing the the frequency of reticuloruminal contractions (Arai *et al.*, 2019; Braun *et al.*, 2002). In the study by Arai et al. (2019), four non-lactating Holstein cows were used to assess the impact of xylazine on ruminal motility using a bolus-type wireless sensor. The study demonstrated that the administration of xylazine hydrochloride at 0.05 mg/kg significantly decreased ruminal motility. Measurements from both a force transducer and the bolus sensor indicated a notable reduction in the amplitude and frequency of ruminal contractions, beginning 10-40 minutes post-administration, which illustrates xylazine's effect on inducing ruminal atony.

Acid-base imbalance, blood glucose and hormonal control

Some other factors less frequently reported can affect reticuloruminal motility. Inhibition of the reticuloruminal motility can be exerted by modification of blood pH, electrolyte disbalances and hyperglycaemia (Constable *et al.*, 2017). Gastrin and cholecystokinin can reduce DMI, as seen in some intestinal nematode infections and, as a consequence, reduce reticuloruminal motility (Foster, 2017).

Factors increasing reticuloruminal motility

Hypermotility is less commonly seen than hypomotility; however, increased motility is often seen in the initial phases of vagal indigestion (Foster, 2017). Some drugs, such as neostigmine, can increase reticuloruminal motility and the strength of contractions.

Table 1- 1 Summary table of the excitatory and inhibitory factors on reticuloruminal motility (adapted from Diseases of the Alimentary Tract–Ruminant. In Constable P, Kenneth W, Hinchcliff W, Stanley H and Grünberg W 2017. Veterinary Medicine, Elvene, pp 436–621. Ed W.B. Saunders. Elsevier).

Afferent input		*Effect on reticuloruminal motility*
Excitatory inputs: low threshold reticular tension receptors	• Increased reticular tension (post-feeding; mild ruminal tympany) • Decreased reticular tension (starvation; anorexia) • Lesion medial wall reticulum (fibrosis due to reticuloperitonitis) • Abomasal acid receptors (Increased activity due to abomasal emptying) • Buccal receptors (post-feeding; inhibitory inputs)	• Increased frequency, duration and amplitude of the primary cycle • Decreased frequency, duration and amplitude of primary contractions • Cause hypomotility and, in some cases, atony; in some other cases, erratic hypermotility • Increase primary cycle activity • Increased reticulorumen activity
High-threshold reticular tension receptors	• Peak of reticular contraction • Severe ruminal tympany • Ruminal impaction with forages	• Depression of primary cycle and ruminal hypomotility
Abomasal tension receptors	• Impactation • Distension or displacement of the abomasum	• It may result in complete ruminal hypomotility
Pain	• Distended abomasum or intestine • Severe pain from other body part • Reticuloperitonitis	• Moderate to complete reduction of the reticuloruminal movements • The degree of inhibition from other body parts depends on the intensity of the pain
Depressant drugs	• Anaesthetics • Central nervous system depressant • Prostaglandin	• Inhibition of primary and secondary cycles resulting in ruminal tympany
Changes in rumen content	• Marked decrease (<5) ruminal pH • Marked increase (>8) ruminal pH • Chemical poisoning	• Inhibition of primary and secondary cycles
Changes in body water, electrolytes and acid-base balance	• Hypocalcemia • Dehydration and electrolyte losses • Acidosis • Alkalosis	• Inhibition of primary and secondary cycles resulting in ruminal tympany
Toxemia/fever	• Peracute coliform mastitis • Acute bacterial penumonia	• Inhibition of primary and secondary cycles
Ruminal distension	• Early ruminal tympany	• Increased frequency of secondary cycle
Covering of cardia (fluid or form)	• Ruminal tympany • Recumbent animal	• Mechanical obstruction resulting in ruminal tympany

1.4 Method of assessment of the reticuloruminal motility

1.4.1 Introduction

The assessment of reticulorumen motility is essential in bovine health as the variations in the reticuloruminal pattern is a critical indicator of cattle health. Reticuloruminal contractions can be assessed in two main ways: invasively or non-invasively, depending on whether surgery is needed or not to implant devices to measure the contraction pattern. Two main types of measurements can be assessed: the inter-contraction interval (ICI), measured as the period of observation (seconds) divided by the number of contractions and the contraction duration (CD), measured as the length of action (seconds) of each contraction.

1.4.2 Invasive methods of recording

Kymography

The first scientific attempts to comprehend the mechanism of reticuloruminal motility date back to two centuries ago. One of the first studies was reported by Flourens in 1833 in fistulated cows, followed by a similar experiment in 1854 (Braun and Schweizer, 2015). Another step was made in 1875 by Toussaint using kymography, and the first complete study was done in 1926 by palpation, observation and pressure recordings (Braun and Schweizer, 2015).

Electromyography

Electrical transducers have successfully recorded reticuloruminal motility in sheep and cows when applied both inside (Reid and Titchen, 1959) and outside of the forestomachs (Dziuk and Sellers, 1955; Bell, 1958; Bowen, 1962; Dziuk, 1964; Dziuk and McCauley, 1965). In 1964, Itabashi described deviation from the baseline using electromyography.

Radiotelemetry

Radiotelemetry was first used by Dracy and Kurtenbach (1965), recording pressure changes in the reticulorumen of cows. Radiotelemetry has been replicated recently in a Polish study using sheep as the experimental animals (Wierzbicka *et al.*, 2021).

Pressure devices (balloons or fluid-filled catheters)

Devices measuring pressure in the bovine forestomach have been used in order to identify patterns of contractions (Sellers and Stevens, 1966; Egert-McLean *et al.*, 2019). More recently, an experiment conducted in the Netherlands used water-filled open-tipped catheters to identify rumination, eating, drinking, and sleeping behaviours and comparing them with visual observation in cows (Scheurwater *et al.*, 2021); the pressure device detected rumination with `accuracy, sensitivity and specificity of 0.92,0.97,0.97, respectively.

Most of the studies mentioned above were technically complex and difficult to perform, and similar attempts have found little application after the 1960s; research has progressively moved away from such types of studies and replaced them with non-invasive methods of recording.

1.4.3 Non-invasive methods of recording

Auscultation, Palpation, Visual

Reticuloruminal motility is commonly measured by visual inspection, palpation and/or auscultation. Human observation is a reliable method to assay eating, chewing, rumination, and activity (Bikker *et al.*, 2014). In a study to validate an ear tag for rumination behaviour, two experienced observers analysed the ethogram of cows divided according to the following categories: rumination, eating, resting, and "active". The agreement between observers for the category's rumination, eating, and resting was high. However, for the "active", the agreement had less strength, indicating a certain level of complexity in the differentiation of activity levels (Bikker *et al.*, 2014). In a study conducted in the Netherlands, using cows, a 3D vision system was allocated on the frame of an automatic milking robot to evaluate the contractions of the rumen by the movement of the left paralumbar fossa (oscillations) and compared with ruminal palpation by a trained assessor immediately after the camera record (Song *et al.*, 2019)**;** the similarity between oscillations detected automatically and reticuloruminal contractions identified manually was found to be 0.97.

Diagnostic imaging

Techniques for diagnostic imaging, including radiography and ultrasonography, have been utilised to assess reticuloruminal motility. For instance, in the previous century, several authors carried out radiological studies; however, these studies were eventually discontinued due to a lack of practicality (Phillipson, 1939; Dougherty and Habel, 1955). Ultrasonography has been described as a valid method to measure the bovine biphasic contractions of the reticulum (Braun and Schweizer, 2015). Bovine reticuloruminal motility has been evaluated by ultrasonography after administration of some drugs (Ruckebusch, 1983); for example, metoclopramide has shown a mild and transient prokinetic effect on the rumen (El-Khodery and Sato, 2008). In another study, the negative effect of scopolamine, xylazine, and atropine on reticuloruminal motility was investigated, and contractility was measured by ultrasound (Braun *et al.*, 2002).

Although the methods above are valid for assessing reticuloruminal motility, they are time-consuming and resource-intensive and do not allow for a simultaneous measurement of several cows (Schirmann *et al.*, 2009)**.** For these reasons, industry and academia have focused on alternative devices to assess reticuloruminal motility, such as sensors equipped with triaxial accelerometers.

1.4.4 Devices equipped with triaxial accelerometers measuring reticuloruminal motility

General information about accelerometers

Precision livestock farming (PLF) has gained interest as a useful tool to assess health and welfare in real time to improve animal management. According to a recent systematic review, 129 sensors (from 67 providers in 21 nations) are available commercially, such as accelerometers, accelerometer-based systems, cameras, load cells and miscellaneous milk sensors (Stygar *et al.*, 2021). In particular, accelerometers have been successful and have been applied to many different types of sensors due to their low cost, small size and robustness (Knight, 2020). Generally speaking, the technology uses a piezoelectric sensor that creates a voltage signal directly related to the change of acceleration of the device (Hendriks *et al.*, 2020)**.**

An accelerometer can function either in 2D, measuring acceleration along two axes (X and Y), or in 3D, where it records acceleration across three axes: X, Y, and Z (Hendriks *et al.*, 2020). Typically, accelerometers generate an analog voltage signal representing acceleration, which is then transformed into digital values through an analogue-to-digital converter (ADC). Acceleration is measured in units like meters per second squared (m/s^2) or 1 gram is equivalent to 9.81 m/s^2.

Accelerometer data processing

Accelerometers are commonly used in various applications, including fitness trackers, industrial machinery, etc. The livestock sector is no exception, and an accelerometer has been widely used to collect data to evaluate a wide range of animal behaviours such as eating, rumination, lying time and oestrus behaviour (Braun & Rauch, 2008; Konka *et al*., 2014; Schweinzer *et al.,* 2019 ;Tucker *et al*., 2021). The processing techniques of the algorithms depend on the specific aspect of the animal behaviour measures and the final objectives of the application. Key steps involved in accelerometer data processing are data collection, signal preprocessing, sensor fusion, feature extraction, data segmentation, pattern recognition, interpretation and data visualisation, real-time preprocessing.

Accelerometers are capable of producing a continuous flow of data, capturing the dynamics of acceleration over time (Cook, 2022). This data is gathered using specialised hardware sensors that are integrated with a triaxial accelerometer. These sensors are predominantly wearable, such as neck collars, ear tags, or pedometers, or they can be internally placed, like indwelling reticuloruminal boluses (Stygar *et al.*, 2021). A recent comprehensive review has indicated that in studies involving ruminants, the sampling frequencies typically range from 0.02 to 200 Hz. Most studies, however, use a frequency below 20 Hz, with a median frequency around 12 Hz (Riaboff *et al.*, 2022). The importance of sampling frequency lies in the balance between capturing detailed, accurate data, managing the volume data collected as well as devices' battery life (Riaboff *et al.*, 2022).

Raw accelerometers can contain artefacts or noise data from various sources, such as unwanted vibrations, electrical interference and sensor imperfections (Riaboff *et al.*, 2022). To distinguish real data from noise, one method involves visual inspection by charting the data and looking for anomalies or inconsistencies; noise typically manifests as erratic fluctuations, while true data displays a consistent trend or pattern. The signal-to-noise ratio (SNR) can also be used to compare the expected signal level and the SNR of the sensor used (Riaboff *et al.*, 2022). Preprocessing techniques include the following filtering methods: low-pass filter (attenuating high frequencies), high-pass filter (attenuating low frequencies), or band-pass filters (allowing a specific range of frequencies while attenuating frequencies outside the "bandwidth"), cutoff frequencies (determine a specific point at which the filter start). In numerous applications, the combination of various sensors, such as accelerometers, gyroscopes, and magnetometers, enhances accuracy and yields more comprehensive information. Algorithms for sensor fusion integrate data from these diverse sensors to determine orientation and position precisely (Aguilar-Lazcano *et al.*, 2023). Relevant features are extracted from the preprocessed data to capture specific information or patterns. Features include peak acceleration, frequency components, energy, and statistical measures (mean, variance, etc.) (Riaboff *et al.*, 2022). Accelerometer data is often segmented into smaller time intervals or windows to focus on specific activities and/or events; segmentation can be used to identify and analyse particular motions or actions within the data set (Riaboff *et al.*, 2022).

Machine learning and pattern recognition techniques can be applied to identify and classify specific activities or behaviours based on particular features and segmented data (Borchers *et al.*, 2017). Classification algorithms, such as support vector machines (SVMs), decision trees, or deep learning models, are the most commonly adopted (Martiskainen *et al.*, 2009; Scheurwater *et al.*, 2021). The dataset is first split into training and test sets to develop a classification model; subsequently, a model training phase and a model validation phase are necessary to complete the process (Riaboff *et al.*, 2022).

The processed accelerometer data is interpreted in the context of the specific application. For example, in dairy cows equipped with neck collars or ear tags, the processed data might be used to determine the rumination and eating pattern activity at both cow and herd levels (Chapa *et al.*, 2020). Data visualisation techniques, such as time-series plots, histograms, heat maps, and density plots, provide insights into the processed accelerometer data. Visualisations can aid in understanding trends, anomalies, and patterns in the data (Riaboff *et al.*, 2022).

In some applications, real-time accelerometer data processing is used for immediate feedback (Eckelkamp and Bewley, 2020); one of the most commonly used in the dairy sector is identifying oestrus pattern behaviour; this is commonly used to inseminate cows in the right window (Schweinzer *et al.*, 2019).

Main devices equipped with a triaxial accelerometer measuring bovine reticulorumen motility (indwelling boluses, neck collars, ear tags and noseband sensors)

Indwelling boluses

Reticuloruminal motility has been evaluated by Hamilton et al. (2019) by the use of indwelling reticular motion-sensitive boluses. The technology applied to each was based on a triaxial accelerometer with a real-time clock, an SD card for data storage, and a lithium battery with a battery life of 30 days. Each bolus with all the components weighed 200 g to allow the device to lie permanently on the reticulum. The device measured the inter-contraction time and the amplitude of the presumed contractions (Hamilton *et al.*, 2019). Other prototypes have been used to measure reticuloruminal motility after xylazine administration (Arai *et al.*, 2019).

Neck collars

In a study conducted in Scotland, a neck collar accelerometer was utilised by a device mounted on the neck to evaluate reticuloruminal contractions. The collar was equipped with a triaxial accelerometer to monitor the muscle's acceleration in correspondence with specific mandible motion (Hamilton *et al.*, 2019)**.** The data showed a robust pattern of time spent ruminating prediction, indicating that the device is a good proxy for reticuloruminal motility (Hamilton *et al.*, 2019). Similarly, an automated system that monitors dry matter intake (DMI) has been developed and validated in the Netherlands. The device used a triaxial accelerometer to detect jaw movements and recognise eating time. A more pronounced circular jaw motion allowed differentiation between eating and rumination (Van Erp-Van der Kooij *et al.*, 2016; Hut *et al.*, 2019). For example, a neck collar with a microphone can measure the regurgitation and rumination sounds with different distinctive sounds in hours registration blocks (Schirmann *et al.*, 2009).

Ear tags and nose band sensors

Some commercial companies have developed ear tags equipped with a triaxial accelerometer to measure different behaviours. The devices have been capable of identifying and measuring one out of six specific behaviours each minute, which include rumination, feeding, resting, low-level activity, standard activity, or high-level activity (Bikker *et al.*, 2014). Similarly, halters equipped with triaxial accelerometers have been used to monitor time spent ruminating (Lee and Seo, 2021). Despite the number of devices commercially used, no comparative studies about accuracy (i.e. ear tags versus indwelling boluses) have been carried out to the best of our knowledge.

1.5 Reticuloruminal motility is a useful indicator of the health of cattle

Eating and chewing are vital in dairy cows' feed intake and production efficiency. Lactating dairy cows spend about 4.5 h/d eating (range: 2.4–8.5 h/d) and 7 h/d ruminating (range: 2.5–10.5 h/d), with a maximum total chewing time of up to 16 h/d (Beauchemin, 2018). Another study reported that the mean rumination time was 436 min/d, ranging between 236 and 610 min/d (White *et al.*, 2017). Different studies have demonstrated that time spent ruminating is linked with clinical and subclinical disease using the magnitude of alterations in rumination patterns (Liboreiro *et al.*, 2015; Kaufman *et al.*, 2016). More recently, Perez *et al.*, 2023 compared an automatic health monitoring system based on time spent ruminating and activity monitoring to an intensive clinical examination to evaluate health disorders such as Retained placenta, Metritis, Displaced Abomasum, Ketosis and Mastitis. It was concluded that the automatic health monitoring system was an effective alternative to traditional clinical examination methods, as long as it is supplemented with visual observations to identify any cows the automated system might miss.

Specific diseases affecting reticulorumen motility

Disease detection is one of the most important aspects of sensors applied to the cattle sector. Numerous research projects have evaluated sensors like neck collars, indwelling boluses, and halters for detecting diseases. In these studies, the duration of time spent ruminating as recorded by neck collars has frequently been utilised as an indicator of health status. Conversely, there have been relatively few studies where indwelling boluses have been assessed for disease detection purposes.

The pattern of the reticuloruminal contractions have been used to evaluate early post-partum diseases (i.e. hypocalcaemia), other common cattle diseases (i.e. acidosis) and physiological behaviour useful for management (i.e. parturition). Below are some of the most common diseases affecting reticuloruminal motility in cattle and some of the studies involving their monitoring through sensors.

Hypocalcaemia

Hypocalcaemia is defined as blood calcium concentration below physiological ranges (<2 mmol/L or 8 mg/dL). Calcium plays a very important role in muscle homeostasis as part of the C-calmodulin complex, which initiates a muscle contraction; in the reticulorumen, smooth muscle contraction stops completely if calcaemia falls below 5.3 mg/dL (Jorgensen *et al.*, 1998). In a study by Iowa University, the effect of subclinical and clinical hypocalcaemia in cows fed with different DACD diets was measured using time spent ruminating by neck collars equipped with a triaxial accelerometer (Goff *et al.*, 2020); rumination in the first two days after calving was highly correlated with cow's plasma Calcium concentration. Normocalcaemic cows spent more time ruminating than cows with subclinical hypocalcaemia; in cows with clinical hypocalcaemia, rumination was almost undetectable. On the other hand, no published report is available to assess hypocalcaemia by indwelling boluses. Further research is needed for clinical and subclinical hypocalcaemia to use indwelling boluses; in particular, boluses might offer a more detailed characterisation of inter-contraction intervals and contraction durations, as opposed to relying solely on time spent ruminating.

Metritis

Metritis is defined as an abnormally enlarged uterus in the first 21 days post-partum; severity (mild or severe) changes according to the quantity of purulent material present and other systemic clinical signs. Changes in time spent ruminating have been studied with cows affected by metritis. A study of 2021 demonstrated that cows (equipped with neck collars) affected by metritis spent less time ruminating than healthy cows (Merenda *et al.*, 2021). In another study, the relationship between time spent ruminating and severity of metritis was studied; when cows experienced severe metritis, including systemic signs such as fever. A marked reduction in time spent ruminating was observed, whereas, in cows with mild metritis, the reduction in time spent ruminating time was much less consistent (Stangaferro *et al.*, 2016a). There are no published reports assessing indwelling boluses for this particular disease, and further research is warranted for this type of device.

Clinical Mastitis

Clinical mastitis is an inflammatory response of the udder, causing visibly abnormal changes in milk. Gram-negative pathogens such as *E. coli* can create an endotoxemia state in cows, which can affect the reticuloruminal motility. In contrast, other pathogens are less able to create such a negative effect. Time spent ruminating was evaluated in cows affected by subclinical and clinical mastitis. In a study in the United States, cows equipped with ear tag sensors with clinical mastitis showed a reduction in time spent ruminating compared to healthy cows (Rial *et al.*, 2023). Similarly, in one study conducted in Lithuania using a band nose sensor, a reduction of 61% of time spent ruminating was observed in cows affected by subclinical mastitis compared to healthy cows (Antanaitis *et al.*, 2022). Indwelling boluses have been employed to monitor changes in reticuloruminal temperature due to experimental infection with *Streptococcus uberis* (Rodriguez *et al.*, 2023). The study revealed that increases in reticuloruminal temperature, prompted by intramammary challenges, serve as an indicator for the onset of clinical mastitis in dairy cows; however, further investigation into its effectiveness across various pathogens was suggested.

Lameness

The clinical manifestation of hindered movement is defined as lameness, as described by O'Leary et al. (2020). The impact of lameness on reticuloruminal motility is typically caused either directly by painful sensations or indirectly through a reduction in the frequency of feeding episodes and a lower Dry Matter Intake (DMI). Time spent ruminating using neck collars has been correlated with lameness in different studies. Daily rumination time was reduced in lame cows in the early dry-off (Abuelo *et al.*, 2021). A Dutch study used a neck collar to evaluate different degrees of lameness, moderately and severely; moderately lame cows showed a reduction of 20 and 40 minutes per day, respectively (Hut *et al.*, 2021). No published reports are available for lameness and indwelling boluses at the date of this thesis. Characterisation of the reticuloruminal motility and different types of lameness (for example, claw horn lesions versus infectious lesions) can be particularly interesting for future research.

Displaced abomasum

Displaced abomasum (DA) commonly affects dairy cattle, and negative repercussions on cows' health and production are well described. DA occurs when an excessive amount of gas accumulates in the abomasum in concomitance with low organ motility. A severe reduction of reticuloruminal motility is observed when an episode of DA occurs, with the study reporting a mean of 1.40 to 1.13 contractions during 3 minutes of clinical examination, compared with a normal range of 3 every 3 minutes (Wittek *et al.,* 2009). Using a nose-band sensor, Braun and collaborators found that rumination is markedly decreased (126 min/d; range: 362-551 min/d) in cows with displaced abomasum. On the other hand, at present, there are no published reports for indwelling boluses assessing DA.

Hyperketonemia (Ketosis)

Hyperketonemia, or ketosis, is a metabolic disease that manifests when ketone bodies (acetone, acetoacetate, beta-hydroxybutyrate) are excessively elevated. Clinical signs are vague, but a decreased DMI is often seen; as a result, this typically leads to a diminished pattern in rumination. For example, in a study conducted in 2016, cows solely affected by ketosis ruminated 25 ± 12.8 minutes/day less than healthy herd mates and cows with ketosis plus a concomitant disease, ruminated about 44 minutes/day less (Kaufman *et al.*, 2016). Similarly, in a study conducted in the USA, the time spent ruminating recorded by neck collar was decreased in hyperketonemia cows by 9.83 min/d ± 6.4 compared with unaffected cows (Abuelo *et al.*, 2021). No published reports are available for indwelling boluses and ketosis at present.

Retained placenta

Retained placenta (RP) occurs when a cow fails to expel the foetal membranes within the first 24 hours after giving birth. Typically, cows with RP exhibit systemic illness, are often characterised by reduced dry matter intake and less time spent ruminating. Liboreiro *et al.* (2015) observed that cows with RP ruminated for shorter periods compared to healthy cows. Additionally, Abuelo and colleagues discovered a notable decrease in rumination time in cows with RP, measured using a neck collar device, as reported in their 2021 study. However, there are no published studies available regarding the use of indwelling boluses in the context of retained placenta.

Ruminal Acidosis

Ruminal acidosis is a depression of ruminal pH; two main forms are described: acute and subacute. In the acute form, the ruminal pH is below 5, leading to a reduction of ruminal motility (Plaizier *et al.*, 2022). Subacute ruminal acidosis (SARA) occurs when a moderate reduction in ruminal pH, between 5.8 or 5.6, is observed. Cows affected by SARA have been shown to have a reduction of reticuloruminal motility (Francesio *et al.*, 2020), although the decrease in reticuloruminal motility is not marked as in the acute ruminal acidosis. Ruminal acidosis is mainly linked to excessive ingestion of feed, rapidly fermentable carbohydrates, or lack of effective fiber, which causes an accumulation of volatile fatty acid (VFA), having an acidogenic effect on pH. It has been reported that cows experiencing acidosis ruminate 1.5 hours less on day 1 after an acidosis episode (Devries *et al.*, 2009). When cows experience acidosis, the onset of rumination post-feeding is delayed despite the normal amount of food being fed (Devries *et al.*, 2009). Time spent ruminating is usually used as a management group tool rather than individual disease detection for acidosis (Devries *et al.*, 2009). In a study conducted, by Huot et al. (2023), in the USA with 110 dairy cows, an indwelling bolus was employed to assess variations in reticuloruminal pH; this method has been proven effective in monitoring episodes of SARA. In another study, Francesio and colleagues (2020) utilised a prototype indwelling bolus to assess the reticuloruminal motility between three different diets: a control diet, a high-starch diet and a high-sugar diet. With both diets, a reduction in the interval between contractions was observed compared to the control diet. On the other hand, the high-starch diet notably increased the amplitude of reticuloruminal contractions, unlike the high-sugar diet, which showed no such increase.

With regard to disease detection, most of the research has been done using neck collars and, to a lesser extent, nose band sensors by comparing the estimated time spent ruminating between sick and healthy cows. Conversely, there are only a few published studies that have used indwelling boluses, mainly focusing on changes in body core temperature. Indwelling boluses could potentially offer a more accurate assessment of reticuloruminal motility since they calculate estimated time based on variations in the acceleration of the reticulum, rather than relying on head or mandible movements. Moreover, indwelling boluses can record at a high frequency (every 30-60 seconds), unlike other devices equipped with triaxial accelerometers. This high-frequency recording, combined with the specific location in the reticulum, allows indwelling boluses to more precisely characterise the interval between contractions and the duration of contractions, thus improving the accuracy in detecting specific disease patterns.

Aims of the experimental study

Despite a wide range of research that has been carried out using the triaxial accelerometer to evaluate reticuloruminal motility, some gaps in research have still to be addressed. With this purpose, the present thesis investigates some of the aspects concerning those gaps. In particular, three main aims were highlighted:

- Firstly, to assess if the observations provided by the indwelling bolus align with RRCR as identified through clinical examination, specifically through auscultation and ultrasound techniques.
- Secondly, to compare the rumination time estimates obtained from the indwelling bolus with those from a collar-based accelerometer.
- Thirdly, to outline the diurnal pattern of RRCR utilising data from the indwelling bolus.

2 Evaluation of an indwelling bolus equipped with a triaxial accelerometer for the characterisation of the diurnal pattern of reticuloruminal contractions

This study has been published in a peer-reviewed journal.

Title: Evaluation of an indwelling bolus equipped with a triaxial accelerometer for the characterisation of the diurnal pattern of reticuloruminal contractions

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2.1 Abstract

This observational study aimed to describe the diurnal pattern of reticuloruminal contraction rate (RRCR) and the proportion of time spent ruminating by cattle, using two commercial devices equipped with triaxial accelerometers: an indwelling bolus (placed in the reticulum) and a neck collar. The three objectives of this study were firstly to determine whether the indwelling bolus provided observations consistent with RRCR as determined by clinical examination using auscultation and ultrasound, secondly to compare estimates of time spent ruminating using the indwelling bolus and a collar-based accelerometer, and finally to describe the diurnal pattern of RRCR using the indwelling bolus data. Six rumen-fistulated, non-lactating Jersey cows were fitted with an indwelling bolus (SmaXtec Animal Care GmbH, Graz, Austria) and a neck collar (Silent Herdsman, Afimilk Ltd. Kibbutz Afikim, Israel), and data were collected over two weeks. Cattle were housed together in a single straw-bedded pen and fed ad libitum hay. To assess the agreement between the indwelling bolus and traditional methods of assessing reticuloruminal contractility in the first week, the RRCR was determined over 10 min, twice a day, by ultrasound and auscultation. Mean inter-contraction intervals (ICI) derived from bolus and ultrasound, and from auscultation were 40.4 ± 4.7, 40.1 ± 4.0 and 38.4 ± 3.3 s. Bland–Altmann plots showed similar performance of the methods with small biases. The Pearson correlation coefficient for the time spent ruminating derived from neck collars and indwelling boluses was 0.72 (highly significant, $P < 0.001$). The indwelling boluses generated a consistent diurnal pattern for all the cows. In conclusion, a robust relationship was observed between clinical observation and the indwelling boluses for estimation of ICI and, similarly, between the indwelling bolus and neck collar for estimating rumination time. The indwelling boluses showed a clear diurnal pattern for RRCR and time spent ruminating, indicating that they should be useful for assessing reticuloruminal motility.

2.2 Introduction

Ruminant digestive physiology has been widely studied because information about forestomach motility can be used as an overall indicator of cattle health (Grünberg and Constable, 2009). The forestomach motility largely depends on the contractions of the first two forestomachs of cattle: the reticulum and the rumen, also referred to as the reticulorumen (Grünberg and Constable, 2009). There are three main reticuloruminal contraction patterns: primary, secondary and rumination (Beauchemin, 2018). Primary contractions are responsible for mixing the ingesta, and begin with the reticulum's biphasic contraction to subsequently involve the rumen in a craniocaudal order (Foster, 2017). Secondary contractions are associated with the eructation process and occur independently of the primary contractions (Foster, 2017). Rumination refers to the process in which a bolus of ingesta is regurgitated from the reticulorumen, re-masticated, re-insalivated and finally re-swallowed (Beauchemin, 2018). An additional reticular contraction preceding the normal biphasic contraction of the reticulum is necessary for rumination to proceed (Beauchemin, 2018). For this reason, the term reticuloruminal contraction rate (RRCR) refers to the complete reticuloruminal contraction cycle, including the biphasic contractions occurring in the primary cycle and the extra-reticular contractions occurring during rumination (Sellers and Stevens, 1966). The RRCR transiently increases in frequency and amplitude during eating (Balch, 1952; Ruckebusch, 1993), and decreases during rumination and recumbency (Sellers and Stevens, 1966). Lactating dairy cows spend about 7 h/d ruminating (range: 2.5–10.5 h/d), 4.5 h/d eating (range: 2.4–8.5 h/d) (Beauchemin, 2018). Dairy cows with unrestricted feed access tend to spend less time eating, and they ruminate for a longer period (Beauchemin, 2018). RRCR can be assessed by measuring the frequency of contractions (number of contractions per unit of time) or inter-contraction interval (ICI – time unit divided by the number of contractions). The ICI averages 40–60 s for the primary contractions and 120 s for the secondary contractions (Grünberg and Constable, 2009). Methods for measuring the RRCR are classified as either invasive or non-invasive, depending on whether surgery is required to apply the measuring device (Braun and Rauch, 2008; Han *et al.*, 2022). Invasive methods include electrodes applied in the forestomach to measure electrical activity (Plaza *et al.*, 1996; Wierzbicka *et al.*, 2021) and placement within the reticulum of air- or water-filled pressure devices (Holtenius *et al.*, 1971; Egert-McLean *et al.*, 2019; Scheurwater *et al.*, 2021). Non-invasive methods include ultrasonography and indwelling reticuloruminal boluses, which directly measure reticular movement; less direct non-invasive methods include clinical examination, auscultation and palpation of the paralumbar fossae; however, they cannot differentiate between primary and secondary cycles (Grünberg and Constable, 2009). Previous experimental studies have used prototype indwelling boluses to measure the temperature, pH, ICI, and contraction amplitude of the reticular motility of cows on various diets (Cantor *et al.*, 2018; Arai *et al.*, 2019; Hamilton *et al.*, 2019; Francesio *et al.*, 2020) and to assess the effects of xylazine and atropine (Choi *et al.*, 2020). Similarly, neck collars mounted with accelerometers have been widely used to assess the amount and proportion of time spent ruminating (Konka *et al.*, 2014; Iqbal *et al.*, 2021; Pavlovic *et al.*, 2022). In a recent study conducted in the Netherlands, 5 years of data were collected using neck collars equipped with triaxial accelerometers (Nedap, Groenlo, The Netherlands), demonstrating a distinct diurnal pattern for time spent ruminating (Hut *et al.,* 2019). To the best of our knowledge, no reports have described and characterised the pattern and type of RRCR using a commercial indwelling accelerometer bolus (Han *et al.,* 2022). The three objectives of this study were firstly to determine whether the indwelling bolus provided observations which were consistent with RRCR as determined by clinical examination using auscultation and ultrasound, secondly to compare estimates of time spent ruminating using the indwelling bolus and a collar based accelerometer and finally to describe the diurnal pattern of RRCR using the indwelling bolus data.

2.3 Materials and methods

2.3.1 Animals and experimental procedures

The data were obtained from six rumen-fistulated, adult, non-lactating, non-pregnant Jersey cows aged between 6 and 12 years, on the University of Glasgow research unit (Cochno farm) for 14 d in June 2021, with approval under Home Office Project Licence PP7153972. The cows were 623.5 ± 31.15 kg (mean ± standard deviation). A full clinical assessment of the animals was performed 2 d before the experiment, and no abnormalities were detected in any of the cows. Cattle remained healthy throughout the trial, with no abnormalities. Rumen fistula surgery was performed some years before the present study (2019 for 2 cows and 2013 for 4 cows). Cows were housed together in a single strawbedded pen (~100m2) throughout the study. The total feed fence and water trough lengths were 9 and 1.1m, respectively. No other animals were housed in the shed during the study period. Hay and water were offered ad libitum throughout the study; hay was replenished daily at 7.30–8.00 and 15.45–16.00. The hay was introduced 6 weeks before the trial to stabilise the RRCR, flora and pH (Sellers and Stevens, 1966). Feed analysis was outsourced to an external laboratory (SRUC, Veterinary and Analytical Services, Pentlands Science Park Bush Loan, Penicuik, Midlothian, EH26 0PZ, UK) (Table 2-1). Two devices each equipped with a triaxial accelerometer – an indwelling reticuloruminal bolus (SmaXtec Animal Care GmbH, Graz, Austria), and a neck collar (Silent Herdsman, Afimilk Ltd. Kibbutz Afikim, Israel) – were applied to the six cows. Collars were fitted to cows 6 weeks before the study period and boluses were inserted by a trained technician through the rumen fistulae directly into the reticulum 3 d before starting the study. Cows were individually moved into a crush next to their pen for auscultation and ultrasound examination. The ultrasonographic examination was performed as previously described (Braun and Schweizer, 2015). The sternal region was clipped and contact gel was applied; the ultrasonography was performed using a convex 3.5MHz probe (CTS-900 V, SIUI, China) placed on the ventral paramedian area of the abdomen, to the left of the caudal projection of the xiphoid (Braun and Schweizer, 2015). A contraction was considered to occur when the ventral wall of the reticulum lifted noticeably above the ventral abdominal wall. Simultaneously, a second operator recorded ruminal contractions identified by auscultation of the left paralumbar fossa. The recording period was 10 min/cow and started for both operators when the first RRCR was detected ultrasonographically. The examination was performed twice daily, between 09.00–10.30 and 16 : 30 and 18 : 00 h, for 3 d (Monday, Wednesday, and Friday). The time of sunrise ranged from 04.32 to 04.37 h and sunset from 21.54 to 22.03 h, with an average daylight of 17 h/d. During the second week of the study, collars and boluses were left on the animals and there was no clinical examination to prevent any possible perturbation to the normal diurnal pattern of RRCR.

Table 2-1 Proximate analysis of the hay fed to the cows for all study period.

Parameter	Value
Dry Matter (DM) [1]	736 g/kg
Ash	56.9 g/kg DM
Crude protein [2]	56.9 g/kg DM
ADF	344 g/Kg DM
D value	57.95
ME	8.98 g/kg DM

2.3.2 Data collection

Clinical data were initially recorded on pre-printed paper record sheets and subsequently transferred to a spreadsheet (Microsoft Excel, 2020). Accelerometer data were obtained as plain text files from the commercial web-platforms for each technology and additional, pre-summarised data for reticuloruminal motility were provided by smaXtec. In each case, the raw accelerometer data were filtered and transformed by the commercially protected algorithms of the manufacturing company. For collars, hourly summarised time spent rumination (collar rumination time – CRT, min/h), eating (collar eating time – CET, min/h) were acquired. For the boluses, 10 min summarised time spent ruminating (bolus rumination time – BRT, min/h, from the commercial platform), inter-contraction interval (BICI, seconds) and contraction duration (BCD, seconds) summarised every 30–60 s and supplied directly by smaXtec were gathered and were aggregated to the hour for consistency with the collar data. Time-series data from the devices were filtered to two datasets: hourly summarised bolus and collar data for the entire study period and separately, bolus data corresponding with the 10-min periods of the clinical examinations.

2.3.3 Statistical analysis

Summary statistics of the mean, SD, first, and third percentile were calculated for each variable. The inter-contraction interval (ICI) was calculated from the 10 min period of the clinical examination (ultrasound and auscultation) as ICI = 600 s/number of contractions. Statistical analyses were performed using (R Core Team, 2020), using the ‘ggplot’, ‘tidyverse’, ‘lubridate’, and ‘mgcv’ packages. Distributions were checked. Pearson’s correlation coefficients were calculated and Bland–Altmann plots were generated to compare clinical examination (ultrasound ICI: USICI and auscultation ICI: AUSCICI) variables with bolus contraction intervals (BICI) and to assess the relationship between the rumination and activity indices from neck collars and boluses. A cyclic generalised additive model (GAM) with cow as fixed effect and smoothed time was fitted using the R function 'gam' in the package 'mcgcv', with up to 24 knots, to define the effects of hour of day (diurnal pattern).

2.4 Results

Table 2-2 lists summary statistics for the two data sets obtained from indwelling reticuloruminal boluses and neck collars. For the hourly collar data, 1296 observations were recorded for the entire study period and 99907 data points were obtained from indwelling boluses. Thirty-six and thirty-five 10-min intervals were measured for AUSC and US, respectively. The mean BICI and USICI were 40.4 ± 4.7 and 40.1 ± 4.0, respectively. For data obtained during the 10 min of clinical examination, the Pearson correlation coefficients (R) for BICI and USICI were 0.55 (95% CI 0.31–0.77; $P < 0.001$); for AUSCICI and BICI $R = 0.40$ (95% CI 0.06–0.62; $P = 0.018$); for AUSCICI and USICI $R = 0.69$(95% CI 0.47–0.83; $P < 0.001$). Polyphasic distributions were observed for BICI and BCD. A zero-inflated distribution was observed for some of the parameters recorded: CRT, CET, BRT.

Table 2-2 Summary of the descriptive statistic.

Variable	Number of observations	Minimum	1st Quartile	Median	Mean	SD	3rd Quartile	Max	Mean (h/d*)
AUSCICI [1] Auscultation inter-contraction interval (s)	36	33.3	37.5	37.5	38.4	± 3.3	40	46	NA
USICI [1] Ultrasound inter-contraction interval (s)	35	33.3	37.5	40.1	40.1	± 4.0	42.9	50	NA
BICI [1] Bolus inter-contraction interval (s)	582	24	32	40	40.1	± 8.9	46	47.7	NA
BICI [2] Bolus inter-contraction interval (s)	99907	24	38	44	46.9	± 13.5	40.4	236	NA
BCD [2] Bolus contraction duration (s)	99907	6	7.5	9	9.3	± 1.9	11	15	NA
BRT[2] Bolus rumination time (min/h)	1296	0	9.5	26.2	23.9	± 16.1	35.9	60	9.6
CRT [2] Collar rumination time (min/h)	1296	6	6	22.5	21.4	± 15.4	33	60	8.6
CET [2] Collar eating time (min/h)	1296	0	0	15.8	22	± 21.9	40.5	61.5	8.8

The Bland–Altman plot shows the indwelling reticuloruminal bolus agreement with the ultrasound examination (Fig. 2-1).

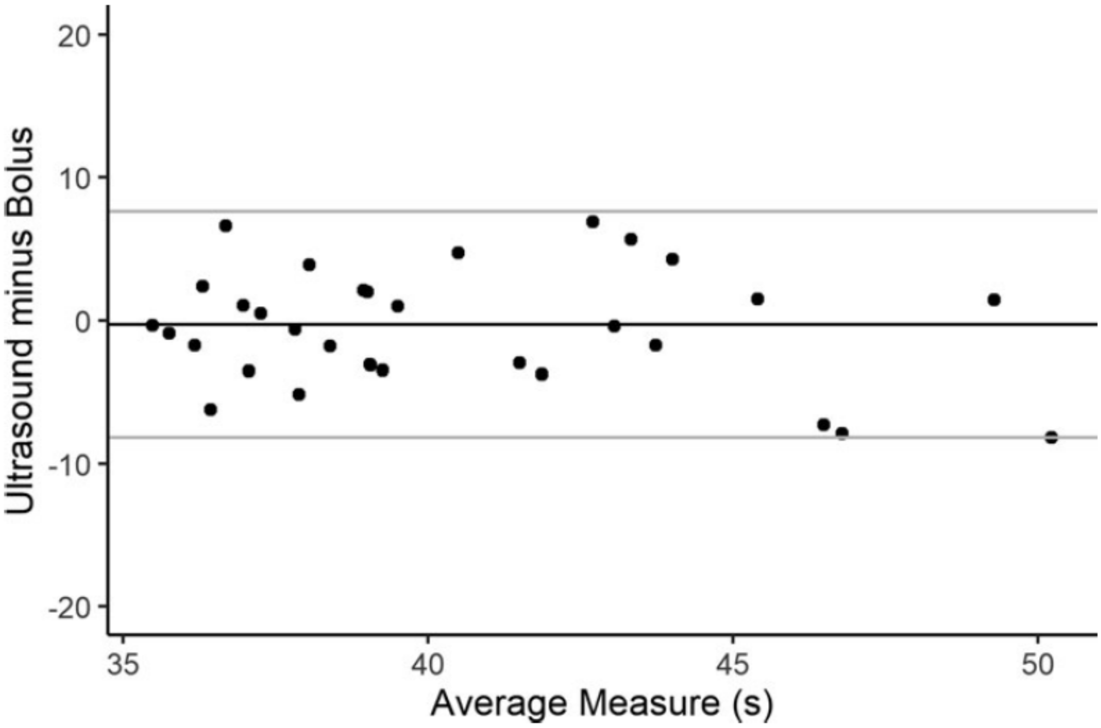

Figure 2-1 Bland-Altman plot agreement for indwelling boluses (smaXtec, Austria) and ultrasonography examination in the 10 min observational period.

Differences between ICI as assessed by ultrasonographic examination and indwelling boluses (USICI − BICI) are plotted against the mean of both estimates. The mean difference (bias) was −0.27 s and the 95% CI for the difference between the observations was −8.2 to 7.6 s. Additional comparisons are given in online Supplementary Fig. 2-2 (ICI values for individual cows measured during the clinical examination) and Fig. 2-3 (the same parameters comparing AM and PM time periods).

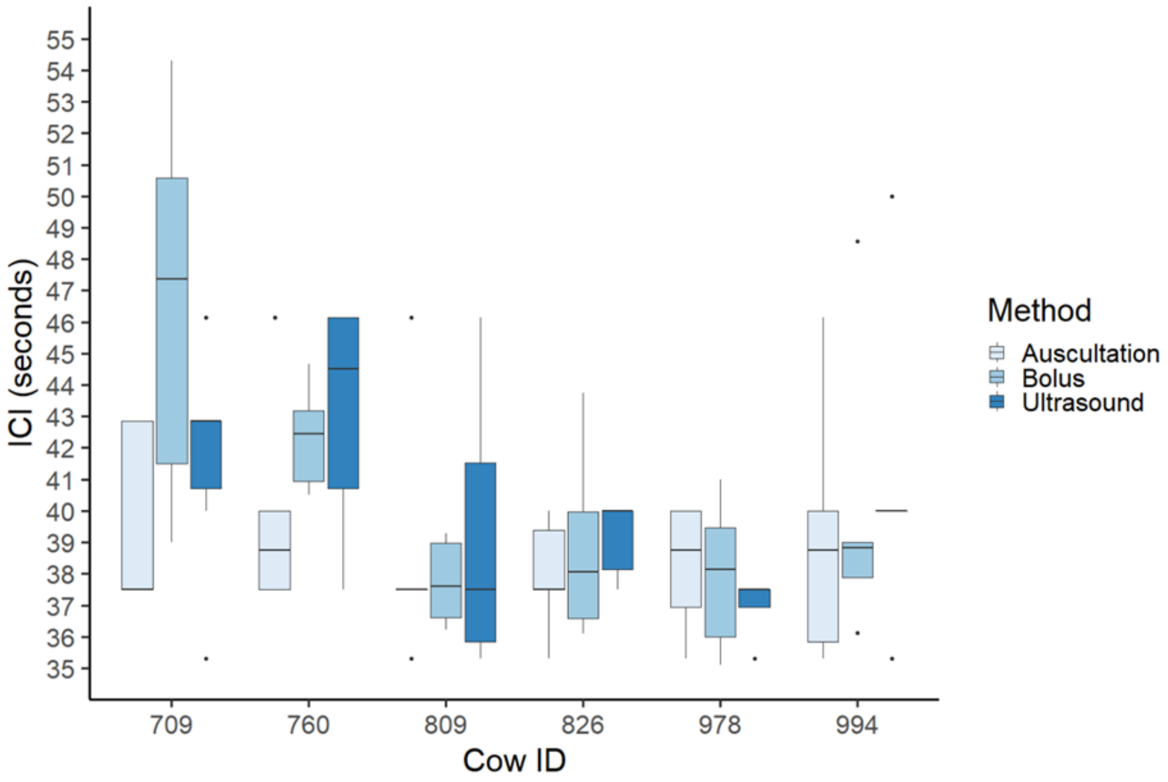

Figure 2-2 The boxplots show the inter-contraction interval (ICI) by cow during the 10 minutes of the clinical examination, as measured by auscultation, ultrasound, and indwelling bolus.

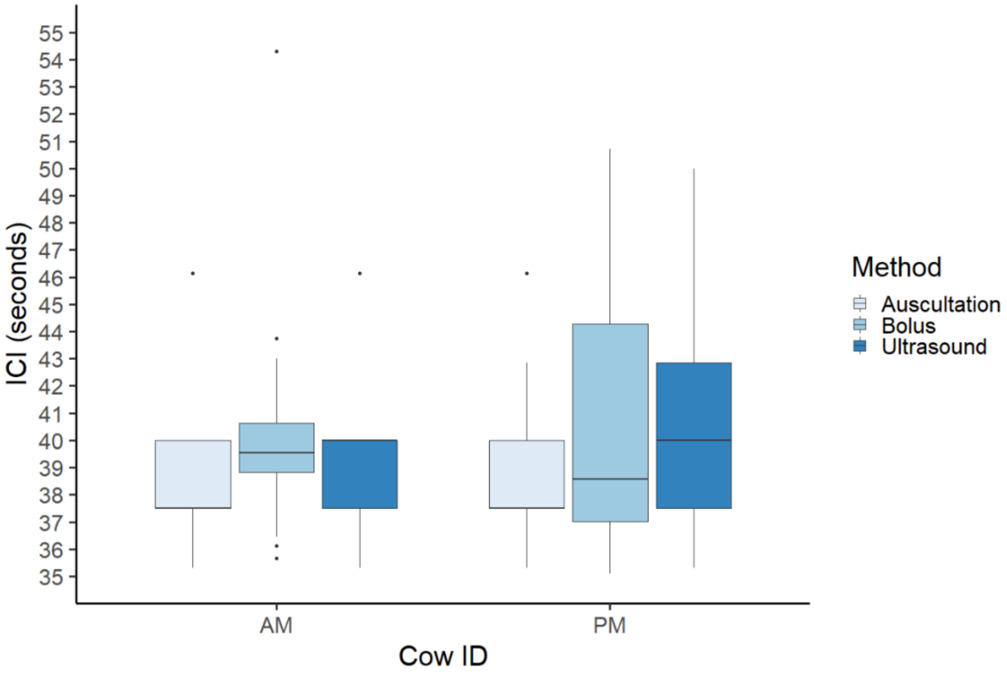

Figure 2-3 Boxplots showing the inter-contraction interval (ICI) in the morning (AM) and afternoon (PM) sessions during the 10 minutes of the clinical examination measured by auscultation, ultrasound and indwelling bolus.

Figure 2-4 shows CRT and BRT for all the cows in the study period within 24 h. The Pearson correlation coefficient for time spent ruminating between the collar and the bolus was 0.72 (CI 95% 0.69–0.74; P < 0.001). The upper boxplot of Fig. 2-4 shows the BRT is at the higher end of its range from midnight to early morning, decreases sharply through the morning, then increases in the middle of the day before falling in the afternoon and increasing again late at night. The pattern for BRT appears congruent with that of the CRT. On average, the time spent eating measured by the collar (CET) was 8.8 h/d, and the eating pattern was approximately the inverse of the time spent ruminating (Figure 2-5).

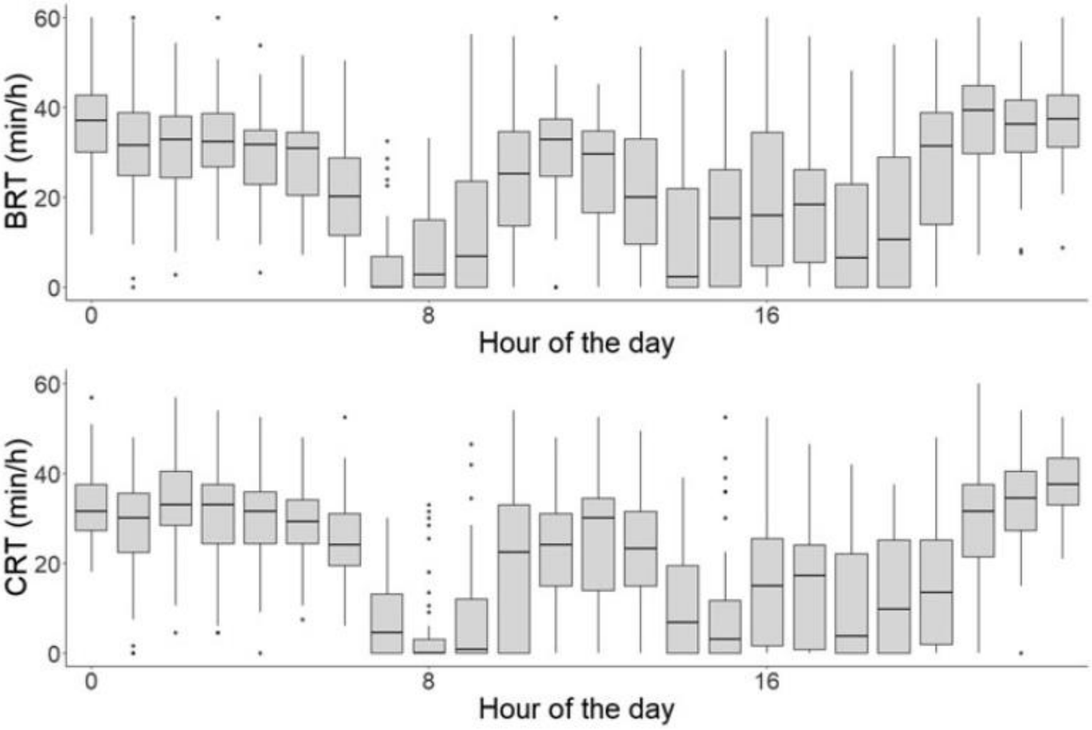

Figure 2-4 A boxplot showing the diurnal pattern of the Bolus Rumination Time (BRT-upper plot) and the Collar Rumination Time (CRT-lower plot), measured in min/h over 24h.

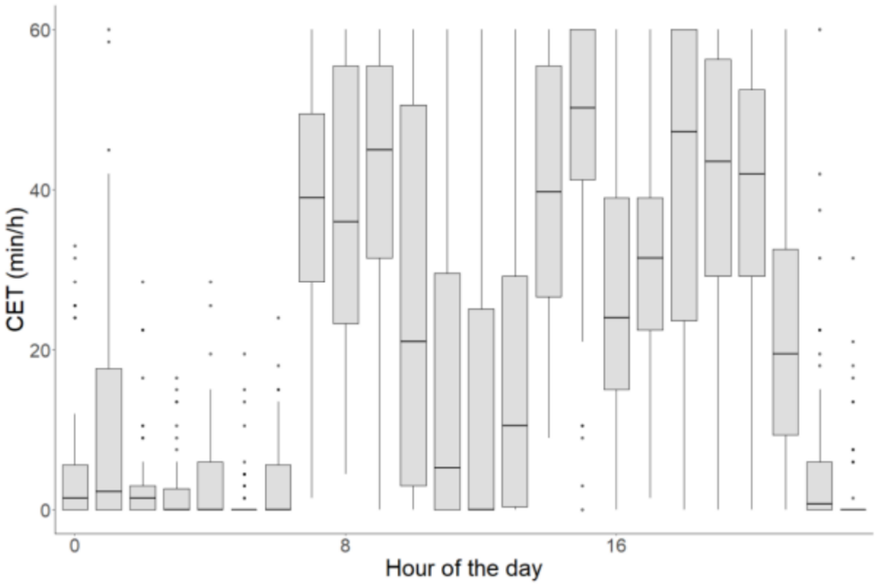

Figure 2-5 A boxplot showing the Collar Eating Time (CET) measured in minutes per hour (24 hours).

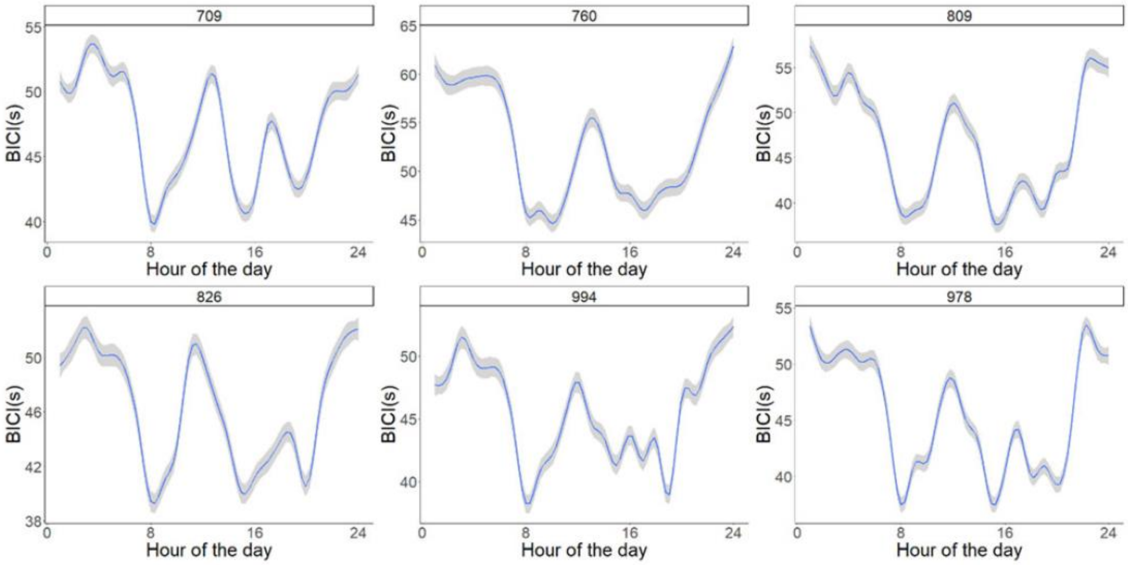

Figure 2-6 Cyclic generalised additive model (GAM) of the diurnal pattern of reticuloruminal inter-contraction interval measured by the indwelling boluses (y-axis) by hour of the day (x-axis), for each cow for data from the entire study period.

Figure 2-6 shows the diurnal pattern of reticuloruminal motility measured by the indwelling boluses for each individual cow.

Table 2-3 Cyclic generalised additive model summary table for the bolus inter-contraction interval (BICI).

Family	**Link function**	**Formula**	**Adjusted R^2**	**Deviance Explained**
Gaussian	Identity	BICI ~s(hour) +CowID	0.14	14 %
		Parametric coefficients		
Factor	**Estimate**	**Std.Error**	**t Value**	**Pr(>t)**
(Intercept)	46.871	0.097	484.422	$< 2 \times 10^{-16}$
Cow 760	5.443	0.141	38.714	$< 2 \times 10^{-16}$
Cow 809	-0.562	0.136	-4.128	3.7×10^{-5}
Cow 826	-0.903	0.136	-6.643	3.1×10^{-11}
Cow 978	-1.742	0.135	-12.875	$< 2 \times 10^{-16}$
Cow 994	-1.577	0.136	-11.639	$< 2 \times 10^{-16}$
		Approximate Significance of Smooth terms		
Factor	**edf**	**Red. df**		**p-value**
S (hour)	21.54	22625.4		$< 2 \times 10^{-16}$

A common diurnal pattern can be seen for all the animals. The cyclic GAM is summarised in Table 2-3: The smoothed effect of time was significant and each cow had a significant effect on BICI. Despite all terms being significant, the model explained only 14% of the variance.

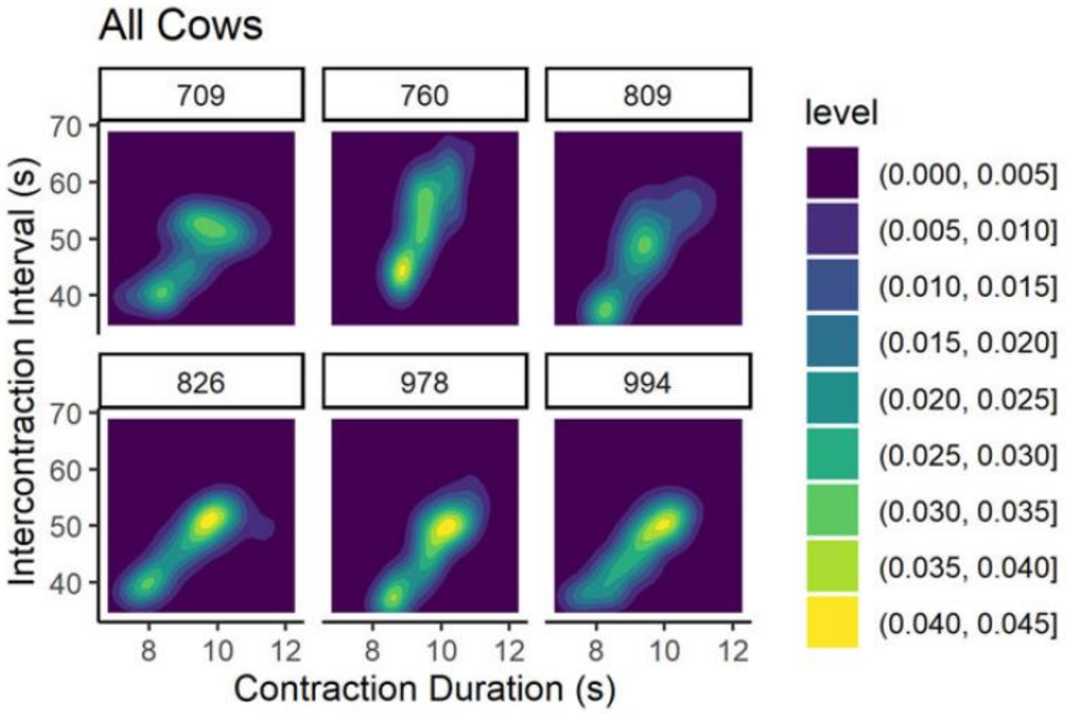

Figure 2-7 Density plot of data from the entire study period showing the bolus inter-contraction interval (BICI) on y-axis and bolus contraction duration (BCD) in the x-axis.

Figure 2-7 shows the relationship between BICI and BCD. Except for Cow 994, all cows showed two peaks in density, a major peak at contraction duration (CD) ~10 s and ICI ~50– 60 s and a minor peak at CD ~8 s and ICI ~40–45 s. Cow 994 showed the same major peak, but the minor peak was less evident.

2.5 Discussion

We used an indwelling bolus and a neck collar, both equipped with a triaxial accelerometer, to characterise the diurnal pattern of time spent ruminating, and the bolus was also used to measure RRCR. The RRCR data provided by the indwelling boluses were consistent with the ultrasonographic examination, and there was an excellent correspondence between the diurnal patterns of the proportion of time ruminating from the indwelling boluses and the neck collars. The first objective of our study was to determine whether the indwelling bolus provided observations on RRCR that were consistent with those obtained from clinical examination using auscultation and ultrasound. Although there is no recognised gold standard to measure RRCR, among the non-invasive methods, ultrasonography has been assessed as a valid method to measure the biphasic contractions of the reticulorumen (Braun and Schweizer, 2015). Braun and Schweizer (2015) visualised the biphasic reticular and rumen atrium contractions of 45 cows over a 9 min observation period, estimating the CD and counting the number of contractions in this period. The CD of the first reticular, the second reticular and the ruminal atrial contractions were 2.0–3.2 s (2.5 ± 0.32), 4.1–6.7 s (5.3 ± 1.02), and 2.2–7.5 s (5.0 ± 0.83), respectively. The number of contractions in 9 min of examination for the first, second reticular, and rumen atrium contractions were 6–17 (11.0 ± 2.12), 6–17 (11.0 ± 2.12), 6–15 (10.7 ± 2.10), respectively. Calculating an ICI from these data suggests values of 49.1 s for the first and the second reticular contractions and 50.5 s for the ruminal atrial contractions. These results are broadly consistent with our study, in which the ICI measured by the indwelling boluses was ~47 s over the entire study. Two prototypes of accelerometer-based bolus have been described previously (Hamilton et al., 2019; Francesio et al., 2020), but only one provided an estimate of ICI of approximately ~51 s (Francesio et al., 2020), using the same cows as were used in the present study. The indwelling boluses used in our study provided information consistent with our clinical observations and with previous investigations. Regardless of our attempts to standardise the clinical observation period in our study, frequency spectrum resolution inevitably introduces potential for error in our clinical estimates of ICI. We attempted to estimate the period between contractions during a finite window of observation, commencing with the identification of a reticular contraction, and continuing for 600 s, meaning that from about 540 s onward, no contraction would be likely to be followed by another recorded contraction. This would likely lead to something like a 5–10% error in our clinical estimates. Frequency spectrum resolution is not commonly discussed in medical and biological sciences, although it is acknowledged as an issue with remote heart-rate estimation, and computational solutions to the problem are dependent on a larger volume of data than our clinical observations (Pan *et al.*, 2022). Regarding the second objective of comparing estimates of time spent ruminating using the indwelling bolus and a collar-based accelerometer, a useful correlation of 0.72 (95% CI 0.69–0.64) was obtained for the time spent ruminating measured by the indwelling boluses and the neck collars, and the patterns of temporal variation were the same. The neck collars (Afimilk Silent Herdsman) were previously shown to identify rumination and eating with a sensitivity of 85%, and an accuracy of 90% (Konka *et al.*, 2014). The diurnal pattern of time spent ruminating measured by the neck collars in our study is consistent with published literature using similar devices: a recent study in the Netherlands evaluated five years of rumination data measured by a neck collar and showed a similar pattern to ours (Hut *et al.*, 2022). The time spent ruminating has been extensively studied (Stangaferro *et al.*, 2016b, c, a; Stevenson, 2022) because variation in time spent ruminating has been associated with subclinical and clinical diseases (Liboreiro et al., 2015). Our third objective was to characterise the diurnal pattern of time spent ruminating, which has previously been evaluated in non-lactating dairy cows with a neck collar (Schirmann *et al.,* 2012). The periods when the proportion of time spent ruminating was highest were between feed deliveries during the day and at night (it was positively associated with lying behaviour: Schirmann *et al.*, 2012). In our study, the indwelling boluses reported similar diurnal patterns of rumination and eating, consistent with the collars, and with previous work. The reticuloruminal motility was also consistent in all six cows. The BICI was shorter in the morning around feeding time, consistent with the literature (Balch, 1952; Braun and Rauch, 2008). Although the hay was offered ad libitum and was replenished twice a day, the influence of this replenishment on the behavioural pattern of reticulorumen contractions cannot be completely ruled out (DeVries *et al.*, 2003). The distributions of CD and ICI were polyphasic, and contour density plots of CD against ICI showed that most cattle had two peaks: a major peak at CD ~10 s and

ICI ~50–60 s and a minor peak at CD ~8 s and ICI ~40–45 s. We propose that these two peaks represent different types of contractions. The primary contraction cycle begins with a biphasic contraction of the reticulum, followed by a contraction which passes through the rumen in a craniocaudal direction. During rumination, a reticular contraction precedes the usual bi-phasic contraction (Ruckebusch, 1993). In the study of Braun and Rauch, 2008, during eating, the ICI was ~39, and the CD ~7.3 s; during resting, the ICI was ~49.5 s, the CD was ~7 s, and during rumination, the CD was 9.4 s, and the ICI is 55 s; comparing our results to this study, the major peaks shown by the indwelling boluses (CD: 10 s, ICI: 50–60 s) appear similar to the rumination peaks of Braun and colleagues, and are consistent with the observations of (Gasteiner *et al.*, 2022). They found that rumination-associated contractions had longer (12 s) CD than feeding associated contractions (7 s). With regard to the minor peak shown in our study, the CD was ~8 s, and ICI was ~40–45 s, which are consistent with those obtained by Braun and colleagues for the eating and resting behaviour patterns. In a recent study, where the RRCR was measured with water filled open-tipped catheter (Scheurwater *et al.*, 2021), the ICI for rumination was around 48 s, and for eating was 34 s; however, the large variation between the behaviours did not allow classification of the patterns by using a set threshold (Scheurwater *et al.*, 2021). The value of neck collar-mounted accelerometers has been demonstrated for the detection of changes in rate of rumination over time. Variation in rumination rate from accelerometers has been used to diagnose disease in cattle (Cook *et al.*, 2021). Our data show similar performance from an indwelling bolus, using the commercially available data. However, it is possible that there is further potential for the bolus device to be exploited to provide precise indications of the ICI and the CD. With this information, it might be possible to achieve earlier and more consistent diagnosis and characterisation of disease states such as parturient hypocalcaemia. The extent to which the estimates of rumination rate are directly linked to the ICI and CD has not yet been made publicly available, if they have been determined. In conclusion, we report a consistent characterisation of the diurnal pattern of RRCR using a commercial indwelling bolus, supporting the use of these devices to assess reticuloruminal motility. Further investigation of the relationship between ICI and CD in health and disease should enable early diagnosis of disease conditions using this technology.

3 Discussion

3.1 How does the current work fit in the current research context?

Indwelling boluses equipped with triaxial accelerometers are a relatively new technology; some attempts have been made with prototypes, but only a few devices have been commercialised (Han *et al.*, 2022). At present, there are no reports that have investigated RRCR using commercial boluses. For the first time, we report the characterisation of the diurnal pattern RRCR by a commercial indwelling bolus. In addition, for the first time, a clear characterisation of two main types of contractions, most based on ICI and CD, has been made with an indwelling bolus device. Based on the present work, the major contraction was highlighted at CD ~10 s and ICI ~50–60 s and a minor peak at CD ~8 s and ICI ~40–45 s.

The present work can be considered a further step in assessing the pattern of RRCR in a continuous and precise way utilising an indwelling device. Although similar results have been obtained in the past, methods and devices used to monitor the contraction pattern relied on an invasive approach (such as kymography or radiotelemetry), or they were not able to continuously monitor the RRCR without a significant amount of time spent assessing the animal (i.e. ultrasonography). A number of devices can estimate the time spent ruminating, most are based on algorithms measuring neck or head movements to predict the behaviours. On the other hand, a device placed in the reticulorumen is likely to create a more specific and frequent pattern of alerts, which results in a more accurate characterisation of the RRCR.

Furthermore, the presented work represents a useful insight into bovine RRCR. Most of the research has been carried out mostly in the first half of the 20th century, and although bovine has always been one of the most relevant livestock species, a good proportion of trials have been carried out using sheep as they allowed an easier surgical approach and somewhat reduced the cost of research.

3.2 Comparative analysis of rumination monitoring devices

Neck collar

The most common devices to evaluate time spent ruminating and eating are mounted neck collars equipped with triaxial accelerometers. They have been widely used to assess and validate rumination by several studies. For example, Ambriz-Vilchis and collaborators (2015) compared the estimate of time spent ruminating by a neck collar and visual observations directly for indoor-housed and grazing cows. The study showed that neck collars could be advised as a valid alternative to visual observation for rumination for indoor-housed cows, but performances for grazing time spent ruminating did not achieve an adequate level of trustability. Conversely, Iqbal *et al.* (2021) found a strong agreement between visual observation and time spent ruminating when neck collars equipped with a triaxial accelerometer were used in a grazing system; similar results were obtained by Werner *et al.* (2019).

Ear tag

In recent years, ear tags equipped with a triaxial accelerometer have become quite popular. Similarly to neck collars, they used head movement as a proxy to estimate time spent ruminating (Beauchemin, 2018; Reiter *et al.*, 2018). Published research about ear tag rumination found the correlation between visual observation and ruminating ranging between 0,69 to 0.97 and a concordance correlation coefficient of 0,59 to 0.96 depending on the brand devices (Bikker *et al.*, 2014; Borchers *et al.*, 2017). In the same studies, there was a lower agreement between visual observation and time spent eating; this was most likely because the characterisation and identification of eating behaviour can vary between individuals. Similar to neck collars, most of the research using ear tags has been conducted for the indoor-housed cow; however, some attempts have also been made for the grazing system. Pereira *et al.* (2020) compared an ear tag-based accelerometer with a noseband sensor for grazing behaviour; a high agreement was observed between the two devices.

Noseband (halter)

Noseband sensor pressure has been used to evaluate time spent ruminating and eating, although they are less popular than ear tags and neck collars. Similarly, to the ear tags and neck collars, most studies focused on simultaneous visual observation and data recording. Braun and colleagues (2015) have widely studied nose band sensors to measure time spent ruminating and eating; the two behaviours can be easily differentiated on the basis of the different characteristic pressure profiles. In the study of Braun and colleagues, a high agreement between visual observation and rumination /eating behaviours was observed for cows in tie stalls. Similarly, Rombach *et al.* (2018) showed good reliability when the sensor-based system was used for the grazing system.

3.3 The future direction of sensor technology

In the last 20 years, the so-called precision livestock sector has expanded dramatically. Several private companies have invested substantial financial resources to develop devices that allow continuous monitoring to improve animal welfare and productivity. In this context, applied research to validate the sensor becomes increasingly important to prove their robustness and suitability (Manning *et al.*, 2021). In their systematic review, Stygar and colleagues (2021) categorised commercially available devices for dairy cattle based on the technology used (such as accelerometers, cameras, etc.) and the type of validation study conducted (self-validation or independent validation). The review found that only 30% of the evaluated accelerometers were validated, and among these, just a handful underwent external assessment. Performances were usually assessed against a gold standard method or the most reliable method of assessment. Visual observation was used to compare the estimated time spent ruminating; alternatively, other validated devices were used to compare and assess the agreement. Most common statistical methods to measure agreement included Pearson correlation coefficient (r), mean bias from the Bland-Altman plots (B-A plots), Spearman's rank correlation coefficient (rs), coefficient of determination (R^2), significance tests for intercept and slope of linear regression (I/S), or concordance correlation coefficient (CCC). Sensors have substituted human labour in many aspects of the cattle farming system due to the continuous and consistent ability to collect data (Michie *et al.*, 2020). A typical example is oestrus detection; the constant monitoring of cow activity is able to pick up more animals in a timely manner rather than human observation, sensibly improving reproductive performances (Michie *et al.*, 2020). On the other hand, accuracy for disease detection (when they are clearly identifiable) is often below acceptable diagnostic standards, with test characteristics most of the time far from the ISO standard of 80% sensitivity and 99% specificity (Hogeveen *et al.*, 2021). For example, a significant drop in time spent ruminating can be associated with a number of diseases and indicates the animal should be further examined (Michie *et al.*, 2020). In this context, gaining a deeper insight into the various contraction types associated with different diseases can aid in pinpointing a particular disease or, more generally, can reveal a distinct RRCR pattern instead of merely indicating a reduction in rumination time. At present, no studies have been published using commercial indwelling boluses and reticuloruminal motility for disease detection. With the present work, further characterisation of the CD and ICI was achieved and the present work can help to pave the way to improve diagnostic accuracy further.

3.4 Farmer expectations for sensor performances

Farmers typically focus on the additional benefits that a sensor can provide, which can be either economic or non-economic. For instance, identifying a higher number of cows in oestrus is seen as an economic benefit because it directly influences the number of artificial inseminations, thereby enhancing fertility rates and ultimately boosting farm productivity (Schweinzer *et al.* 2019). On the other hand, non-economic value is derived from acquiring more precise but not immediately actionable information, such as monitoring the rumination time for each production pen on a dairy farm (Rojo-Gimeno *et al.*, 2019). An important aspect is the sensors' accuracy level with minimal False Positive and False Negative alerts (Lovarelli *et al.*, 2020). For example, false positive alerts can cause excessive labour to verify the event, whereas false negative alerts can fail to detect a particular event, causing mistrust (Hogeveen *et al.*, 2021). Additionally, remote monitoring can help optimise time management and, as a result, improve the quality of life of farmers.

3.5 Safety considerations for monitoring sensors

Safety is a crucial factor when using wearable or indwelling devices on animals, as highlighted by Stygar *et al.* (2021). For example, it is essential to properly fit and routinely inspect neck collars to prevent complications such as constriction in developing animals, like heifers, due to the collars not being adjusted to accommodate their growth. Ear tags should be placed specifically on a part of the ear cartilage to ensure effectiveness and reduce the risk of detachment. Technology has substantially improved in the last two decades, and nowadays, the weight of the devices has decreased over time while maintaining technical recording performances (Beauchemin, 2018).

3.6 Exploring new applications using the indwelling boluses

Sensors are valuable tools in the monitoring of behaviours like lying, sleeping, and drinking in cattle. Lying behaviour can now be accurately and extensively studied using neck and leg collars. The time cows spend lying down (10 to 12 hours a day) is crucial for assessing their welfare. This parameter is influenced by housing, weather, feeding, and social hierarchy. Additionally, lying time correlates with rumination time, as cows often ruminate for several hours while lying, typically during the night (Tucker *et al.*, 2021). However, the exact relationship between the time spent lying and actual sleep duration is not well-defined, with some studies showing no direct correlation (Schirmann *et al.*, 2012). Sleep in cows is divided into non-rapid eye movement (NREM) and rapid eye movement (REM). Sleep deprivation studies in ruminants are rare. One notable study restricted cows from lying down for 14 to 22 hours daily over two months, resulting in a decrease in REM sleep and an increase in NREM sleep (Ruckebusch, 1974). Another study showed that cows deprived of sleep or lying time for 24 hours took 2 to 4 days to recover (Ternman *et al.*, 2012). The connection between sleeping, lying time, and reticuloruminal motility remains largely unexplored; investigating this could reveal reticuloruminal motility patterns that mirror lying and sleeping times, potentially serving as indicators of cattle welfare and productivity (Tucker *et al.*, 2021). Continued research, possibly employing wireless devices like indwelling boluses, is needed to better understand such relationships.

Another physiological behaviour not frequently studied in cattle is drinking. Water plays a vital role in the biological and physical functions of ruminants. In a recent study, a neck collar was used to monitor drinking behaviour using a specific pattern of neck movements; the designed algorithm successfully identified 94% of drinking activities (Williams *et al.*, 2020). Another study utilised decreases in body core temperature, as recorded by indwelling boluses, to infer drinking behaviour (Vázquez-Diosdado *et al.*, 2019). The algorithm developed by the researchers, based on the average reticuloruminal temperature of the cows minus one standard deviation, proved effective in pinpointing drinking episodes. Nonetheless, further investigation is required to fully integrate these observations of drinking behaviour into the broader context of monitoring and predicting the health of cows.

There is little research on the pattern of reticuloruminal motility in wildlife ruminants, and it is limited mainly to deer species (*Cervus elaphus*) (Dziuk *et al.*, 1963; Semiadi *et al.*, 1993). In a study conducted in New Zealand, four castrated deer were monitored through a noseband collar for eating and rumination behaviour for 7 days, measuring jaw movement activity (Stafford *et al.*, 1993). The deer spent between 404 to 465 min/day eating and around 378 min/day ruminating. The reticuloruminal motility patterns were similar to those of cattle and sheep, although the number of contractions associated with eructation was lower than in domesticated species (Stafford *et al.*, 1993).

Further research is needed to fully understand the pattern of reticulorumen motility in other wildlife species. Similarly to cattle, once the physiological patterns are understood, the RRCR can be used as a proxy for health. For instance, a recent meta-analysis highlighted the negative impact of gastrointestinal nematodes on sheep, affecting their dry matter intake and growth rate (Méndez-Ortíz *et al.*, 2019). Investigating the link between RRCR and diseases such as gastrointestinal nematodes in wildlife ruminants could provide valuable insights into this relationship.

3.7 Gap in research evaluating reticuloruminal motility using indwelling boluses equipped with a triaxial accelerometer

The thesis offers some insight into the gaps between the use of indwelling boluses and the current literature research. Nonetheless, a wide range of aspects are still needed to be clarified. Perhaps, although technically challenging, a thorough description of reticulorumen motility using simultaneously indwelling boluses and electromyography can give invaluable insight into some more complicated aspects of reticuloruminal motility, such as a clear differentiation of contraction types during sleeping, drinking, ruminating and eating allowing a further characterisation of CD and ICI during these phases.

From animal welfare and farm management, an important gap in the literature exists regarding indwelling boluses and patterns of reticuloruminal motility for disease detection. Although it is very likely that some patterns have been identified and used for commercial purposes, scientific evidence from independent studies is lacking. Such types of studies are needed and can bring clarity to disease detection, enhancing high standards of management.

3.8 Impact of sensor technology on environmental sustainability in farming practices

The attention of public opinion around animal welfare has noticeably increased in the recent past, and consumers require more transparency of on-farm practices, especially when considering large farm units (Manning *et al.*, 2021). A more efficient management of large herds is likely to have a positive effect on carbon footprint (Flysjö *et al.*, 2014). Although sensors equipped with triaxial accelerometers have their own embedded carbon footprint, their application should result in a more efficient farming system (Eory *et al.*, 2015). The continuous monitoring of physiological behaviours should allow farmers to demonstrate not only breaches in the production system but also consistency in good animal health management (Manning *et al.*, 2021). Sensors can enhance animal welfare, leading to a longer lifespan and more effective on-farm breeding replacement strategies, which in turn can reduce the carbon footprint (Rotz and Thoma, 2017). Another positive aspect of sensor application can be represented by the early identification of sick animals; they decrease the efficiency of the farm, leading to an increase in carbon footprint per unit of output (Jonsson *et al.*, 2022). In this context, a deeper comprehension of the pattern of reticulorumen through continuous monitoring can help to improve digestive efficiency, disease detection and optimise feeding strategies. In summary, enhancing the comprehension of reticuloruminal motility patterns can lead to more efficient and sustainable farming practices, which is pivotal in lowering the carbon footprint of farm systems.

4 Conclusion

Central to this study was the evaluation of the diurnal patterns of reticuloruminal motility by indwelling boluses and neck collars equipped with triaxial accelerometers. In particular, by bridging the gap between traditional methods of assessment of reticuloruminal motility and modern sensor technologies, the research conducted for this thesis contributes to the enhancement of the field of precision livestock farming. Another key finding of the present work was the successful application and reliability of sensor technologies in accurately monitoring and characterising the diurnal pattern of reticulorumen motility. Devices like indwelling boluses and neck collars equipped with accelerometers have revolutionised the way these physiological processes are studied, shifting from manual, time-consuming methods to automated, real-time monitoring. This technological advancement has potentially significant implications for early disease detection, allowing for timely interventions that can prevent the escalation of health issues. The integration of sensor technology in farming has opened new avenues for improving animal welfare and farm productivity. Patterns of rumination and other related activities can now be studied in greater detail, providing insights into the physiological responses of cattle to various environmental and managerial practices. By providing continuous and precise data on reticuloruminal activities, these tools enable farmers to make informed decisions about feeding strategies, health management, and overall herd management. The ability to detect subtle changes in an animal's health status can lead to more targeted and effective treatment plans, thereby enhancing the welfare of the animals and the economic viability of farm operations. Despite the significant progress made, the thesis also highlighted the challenges faced in the widespread adoption of sensor technology in cattle farming. Issues related to solid research for the validation of sensors, data interpretation, and the integration of technology into existing farm systems were some of the key barriers identified. Further research should focus on addressing these challenges and exploring the potential of emerging technologies, such as artificial intelligence, in enhancing the full potential of the sensor-based monitoring system. In conclusion, this thesis represents a comprehensive overview of the role of reticuloruminal motility in cattle health and the transformative potential of sensor technologies in monitoring these processes. The findings not only enhance our understanding of bovine physiology but also pave the way for advancements in cattle farming practices that prioritise animal welfare, farm efficiency, and sustainability.

Gaps in the Current Knowledge:

- **Differentiation between contraction types**: The current research predominantly focuses on rumination analysis. A thorough analysis of reticuloruminal motility and specific pattern using indwelling boluses is lacking.
- **Specificity of devices disease detection**: Limited studies focus on the use of indwelling boluses for the direct detection of specific diseases affecting reticuloruminal motility, unlike more common assessments with neck collars or external sensors.

Required Future Research:

- **Enhanced disease detection algorithms:** Develop and validate algorithms that use data from indwelling boluses to directly detect and monitor specific diseases, improving early detection and intervention capabilities.
- **Characterisation of contraction type for physiological behaviours**: Research into advanced signal processing and machine learning techniques to better differentiate and characterise the various types of reticuloruminal contractions, enhancing understanding of rumen health and function.
- **Environmental and management impact studies:** Investigate how different environmental conditions and management practices affect the data collected by

indwelling boluses aiming to adjust algorithms or interpretations according to these factors.

- **Comprehensive health monitoring systems integration**: Explore the integration of data from indwelling boluses and neck collars with other health monitoring technologies and farm management systems to develop a comprehensive, real-time health monitoring platform for dairy herds.

Bibliography

Abuelo A, Wisnieski L, Brown JL and Sordillo LM (2021) Rumination time around dry-off relative to the development of diseases in early-lactation cows. *Journal of Dairy Science* **104**, 5909–5920.

Aguilar-Lazcano CA, Espinosa-Curiel IE, Ríos-Martínez JA, Madera-Ramírez FA and Pérez-Espinosa H (2023) Machine Learning-Based Sensor Data Fusion for Animal Monitoring: Scoping Review. *Sensors* **23**, 1–28.

Ambriz-Vilchis V, Jessop NS, Fawcett RH, Shaw DJ and Macrae AI (2015) Comparison of rumination activity measured using rumination collars against direct visual observations and analysis of video recordings of dairy cows in commercial farm environments. *Journal of Dairy Science* **98**, 1750–1758.

Antanaitis R, Juozaitienė V, Malašauskienė D, Televičius M, Urbutis M, Rutkaukas A, Šertvytytė G and Baumgartner W (2022) Identification of Changes in Rumination Behavior Registered with an Online Sensor System in Cows with Subclinical Mastitis. *Veterinary Sciences* **9**, 1–16.

Arai S, Okada H, Sawada H, Takahashi Y, Kimura K and Itoh T (2019) Evaluation of ruminal motility in cattle by a bolus-type wireless sensor. *Journal of Veterinary Medical Science* **81**, 1835–1841.

Balch CC (1952) Factors Affecting the Utilization of Food by Dairy Cows - The rate of Contraction of the Reticulum. *British Journal of Nutrition* **6**, 366–375.

Barone R and Bortolami R (2014) *Anatomia Comparata Dei Mammiferi Domestici Vol.3. Splancnologia (Apperecchio Digerente e Respiratorio),* pp 289–329. Edagricole.

Beauchemin KA (2018) Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science* **101**, 4762–4784.

Bell FR (1958) Pressure developed within the Reticular Compartment of the Ruminant Stomach. *Nature* **181**, 494–494.

Bell FR and Itabisashi T (1973) The electroencephalogram of sheep and goats with special reference to rumination. *Physiology & Behavior* **11**, 503–514.

Bikker JP, van Laar H, Rump P, Doorenbos J, van Meurs K, Griffioen GM and Dijkstra

J (2014) Technical note: Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. *Journal of Dairy Science* **97**, 2974–2979.

Borchers MR, Chang YM, Proudfoot KL, Wadsworth BA, Stone AE and Bewley JM (2017) Machine-learning-based calving prediction from activity, lying, and ruminating behaviors in dairy cattle. *Journal of Dairy Science* **100**, 5664–5674.

Bowen JM (1962) Effects of insulin hypoglycemia on gastrointestinal motility in the sheep. *American Journal of Veterinary Research* **23**, 948–954.

Braun U and Rauch S (2008) Ultrasonographic evaluation of reticular motility during rest, eating, rumination and stress in 30 healthy cows. *Veterinary Record* **163**, 571–574.

Braun U and Schweizer A (2015) Ultrasonographic assessment of reticuloruminal motility in 45 cows. *Schweizer Archiv Fur Tierheilkunde* **157**, 87–95.

Braun U, Gansohr B and Haessig M (2002) Ultrasonographic evaluation of reticular motility in cows after administration of atropine, scopolamine and xylazine. *Journal of Veterinary Medicine Series A: Physiology Pathology Clinical Medicine* **49**, 299–302.

Byskov M V., Nadeau E, Johansson BEO and Nørgaard P (2015) Variations in automatically recorded rumination time as explained by variations in intake of dietary fractions and milk production, and between-cow variation. *Journal of Dairy Science* **98**, 3926–3937.

Campling RC and Morgan CA (1981) Eating behaviour of housed dairy cows—A review. *Dairy Science Abstracts* 57–63.

Cantor MC, Costa JHC and Bewley JM (2018) Impact of observed and controlled water intake on reticulorumen temperature in lactating dairy cattle. *Animals* **8**, 1–10.

Chapa JM, Maschat K, Iwersen M, Baumgartner J and Drillich M (2020) Accelerometer systems as tools for health and welfare assessment in cattle and pigs – A review. *Behavioural Processes* **181**, 104262.

Choi W, Ro Y, Hong L, Ahn S, Kim H, Choi C, Kim H and Kim D (2020) Evaluation of ruminal motility using an indwelling 3-axis accelerometer in the reticulum in cattle. *Journal of Veterinary Medical Science* **82**, 1750–1756.

Constable P, Kenneth W, Hinchcliff W, Stanley H and Grünberg W (2017) Diseases of the Alimentary Tract–Ruminant. In *Veterinary Medicine*, Elvene, pp 436–621. Ed W.B.

Saunders. Elsevier.

Cook J (2022) Association between Prepartum Alerts Generated Using a Commercial Monitoring System and Health and Production Outcomes in Multiparous Dairy Cows in Five UK Herds. *SSRN Electronic Journal* 1–15.

Cook JG, Pepler PT and Viora L (2021) Association of days in close up, gestation length, and rumination around time of calving with disease and pregnancy outcomes in multiparous dairy cows. *Journal of Dairy Science* **104**, 9093–9105.

Dado RG and Allen MS (1993) Continuous Computer Acquisition of Feed and Water Intakes, Chewing, Reticular Motility, and Ruminal pH of Cattle. *Journal of Dairy Science* **76**, 1589–1600.

Devries TJ, Beauchemin KA, Dohme F and Schwartzkopf-Genswein KS (2009) Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. *Journal of Dairy Science* **92**, 5067–5078.

DeVries TJ, Von Keyserlingk MAG and Beauchemin KA (2003) Short communication: Diurnal feeding pattern of lactating dairy cows. *Journal of Dairy Science* **86**, 4079–4082.

Dougherty RW and Habel RE (1955) The cranial esophageal sphincter, its action and its relation to eurctation in sheep as determined by cinefluorography. *The Cornell Veterinarian* **45**, 459–464.

Dracy AE and Kurtenbach AJ (1965) Radiosonde for Measuring Bovine Intraruminal Reticular Pressures. *Journal of Dairy Science* **48**, 1250–1251.

Dulphy JP, Remond B and Theriez M (1979) Ingestive behaviour and related activities in ruminants. In *5th Int. Symp. Ruminant Physiology.*, pp 103–122. Eds Y Ruckebusch and P Thivend. Berlin, Germany: Springer.

Dziuk HE (1964) Radiotelemetry for stomach motility studies in ruminants. *Medical Electronics & Biological Engineering* **2**, 281–287.

Dziuk HE and McCauley EH (1965) Comparison of ruminoreticular motility patterns in cattle, sheep, and goats. *American Journal of Physiology-Legacy Content* **209**, 324–328.

Dziuk HE and Sellers AF (1955) Physiological studies of the vagal nerve supply to the bovine stomach. 1. Comparison of responses in milkfed and roughage-fed calves, using a chronic intrathoracic vagal electrode technique. *American Journal of Veterinary Research* 411–417.

Dziuk HE, Fashingbauer BA and Idstrom JM (1963) Ruminoreticular pressure patterns in fistulated white-tailed deer. *American Journal of Veterinary Research* 772-783.

Eades SC (1993) Endotoxemia in dairy cattle: role of eicosanoids in reticulorumen stasis. *Journal of dairy science*, **76,** 414–420.

Eckelkamp EA and Bewley JM (2020) On-farm use of disease alerts generated by precision dairy technology. *Journal of Dairy Science* **103**, 1566–1582.

Egert-McLean AM, Sama MP, Klotz JL, McLeod KR, Kristensen NB and Harmon DL (2019) Automated system for characterizing short-term feeding behavior and real-time forestomach motility in cattle. *Computers and Electronics in Agriculture* **167**, 105037.

El-Khodery SA and Sato M (2008) Ultrasonographic assessment of the reticular motility in cows after administration of different doses of metoclopramide and neostigmine. *Veterinary Research Communications* **32**, 473–480.

Eory V, Macleod M, Topp CFE, Rees RM, Webb J, McVittie A, Wall E, Borthwick F, Watson C, Waterhouse A *et al.* (2015) Review and update the UK Agriculture Marginal Abatement Cost Curve to assess the greenhouse gas abatement potential for the 5th carbon budget. Final report submitted for the project contract "Provision of services to review and update the UK agriculture M. 274.

Van Erp-Van der Kooij E, Van De Brug M and Roelofs JB (2016) Validation of Nedap Smarttag Leg and Neck to assess behavioural activity level in dairy cattle. *Procceding Precision Dairy Farming* 321–326.

Flysjö A, Thrane M and Hermansen JE (2014) Method to assess the carbon footprint at product level in the dairy industry. *International Dairy Journal* **34**, 86–92.

Foster D (2017) Disorders of Rumen Distension and Dysmotility. *Veterinary Clinics of North America - Food Animal Practice* **33**, 499–512.

Francesio A, Viora L, Denwood MJ, Tulley W, Brady N, Hastie P, Hamilton A, Davison C, Michie C and Jonsson NN (2020) Contrasting effects of high-starch and high-sugar diets on ruminal function in cattle. *Journal of Dairy Research* **87**, 175–183.

Gasteiner J, Fasching C, Astl M and Steinwidder A (2022) Telemetric and long term measurement of reticuloruminal motility - Precision and accuracy of derived rumination time. In *31st World Buiatri Congress*, pp 186–187. Madrid.

Goff JP, Hohman A and Timms LL (2020) Effect of subclinical and clinical hypocalcemia and dietary cation-anion difference on rumination activity in periparturient dairy cows. *Journal of Dairy Science* **103**, 2591–2601.

Gordon JG (1965) The effect of water deprivation upon the rumination behaviour of housed sheep. *The Journal of Agricultural Science* **64**, 31–35.

Gregory PC (1987). Inhibition of reticulo-ruminal motility by volatile fatty acids and lactic acid in sheep. *The Journal of physiology*, *382*, 355–371.

Grünberg W and Constable PD (2009) Function and Dysfunction of the Ruminant Forestomach. In *Food Animal Practice*, 5th ed, pp 12–19. Eds DE Anderson, M Rings and EJ Abrahamsen. St. Louis, Missouri: Saunders Elsevier.

Hamilton AW, Davison C, Tachtatzis C, Andonovic I, Michie C, Ferguson HJ, Somerville L and Jonsson NN (2019) Identification of the rumination in cattle using support vector machines with motion-sensitive bolus sensors. *Sensors (Switzerland)* **19**,.

Han CS, Kaur U, Bai H, Reis BR dos, White R, Nawrocki RA, Voyles RM, Kang MG and Priya S (2022) Invited review: Sensor technologies for real-time monitoring of the rumen environment. *Journal of Dairy Science* **0**, 6379–6404.

Hendriks SJ, Phyn CVC, Huzzey JM, Mueller KR, Turner SA, Donaghy DJ and Roche JR (2020) Graduate Student Literature Review: Evaluating the appropriate use of wearable accelerometers in research to monitor lying behaviors of dairy cows. *Journal of Dairy Science*.

Hogeveen H, Klaas IC, Dalen G, Honig H, Zecconi A, Kelton DF and Sánchez Mainar M (2021) Novel ways to use sensor data to improve mastitis management. *Journal of Dairy Science* **104**, 11317–11332.

Holtenius P, Jacobsson SO and Jonson G (1971) A Method for Recording the Motor Activity of the Reticulum in Cattle. *Acta Veterinaria Scandinavica* **12**, 313–324.

Huot F, Claveau S, Bunel A, Santschi DE, Gervais R and Paquet R (2023) Relationship between farm management strategies, reticuloruminal pH variations, and risks of subacute ruminal acidosis. *Journal of Dairy Science* **106**, 2487–2497.

Hut PR, Mulder A, van den Broek J, Hulsen JHJL, Hooijer GA, Stassen EN, van Eerdenburg FJCM and Nielen M (2019) Sensor based eating time variables of dairy cows in the transition period related to the time to first service. *Preventive Veterinary Medicine* **169**, 104694.

Hut PR, Hostens MM, Beijaard MJ, van Eerdenburg FJCM, Hulsen JHJL, Hooijer GA, Stassen EN and Nielen M (2021) Associations between body condition score, locomotion score, and sensor-based time budgets of dairy cattle during the dry period and early lactation. *Journal of Dairy Science* **104**, 4746–4763.

Hut PR, Kuiper SEM, Nielen M, Hulsen JHJL, Stassen EN and Hostens MM (2022) Sensor based time budgets in commercial Dutch dairy herds vary over lactation cycles and within 24 hours. *PLoS ONE* **17**, 1–19.

Iqbal MW, Draganova I, Morel PCH and Morris ST (2021) Validation of an accelerometer sensor-based collar for monitoring grazing and rumination behaviours in grazing dairy cows. *Animals* **11**, 2724.

Ismail ZB, Al-Majali A, & Al-Qudah K (2007). Clinical and surgical findings and outcome following rumenotomy in adult dairy cattle affected with recurrent rumen tympany associated with non-metallic foreign bodies. *American Journal of Animal and Veterinary Sciences*, **2**, 66-71.

Jonsson NN, MacLeod M, Hayward A, McNeilly T, Ferguson KD and Skuce PJ (2022) Liver fluke in beef cattle – Impact on production efficiency and associated greenhouse gas emissions estimated using causal inference methods. *Preventive Veterinary Medicine* **200**, 105579.

Jorgensen RJ, Nyengaard NR, Ham S, Enemark JM and Andersen PH (1998) Rumen Motility During Induced Hyper- and Hypocalcaemia. *Acta Veterinaria Scandinavica* **39**, 331–338.

Kaufman EI, LeBlanc SJ, McBride BW, Duffield TF and DeVries TJ (2016) Association of rumination time with subclinical ketosis in transition dairy cows. *Journal of Dairy Science* **99**, 5604–5618.

Klemm WR (1966) Sleep and Paradoxical Sleep in Ruminants. *Experimental Biology and*

Medicine **121**, 635–638.

Knight CH (2020) Review: Sensor techniques in ruminants: More than fitness trackers. *Animal* **14**, S187–S195.

Konka J, Michie C and Andonovic I (2014) Automatic classification of eating and ruminating in cattle using a collar mounted accelerometer. *Proceedings of the 39th ICAR Session, May 19-23* 1–8.

Lee M and Seo S (2021) Wearable wireless biosensor technology for monitoring cattle: A review. *Animals* **11**, 2779.

Liboreiro DN, Machado KS, Silva PRB, Maturana MM, Nishimura TK, Brandão AP, Endres MI and Chebel RC (2015) Characterization of peripartum rumination and activity of cows diagnosed with metabolic and uterine diseases. *Journal of Dairy Science* **98**, 6812–6827.

Liebich H-G and Kupliec C (2019) Digestive system. In *Veterinary Histology of Domestic Mammals and Birds: Textbook and Colour Atlas*, 5th ed, pp 179–238. Sheffield: 5M Publishing Ltd.

Lovarelli D, Bacenetti J and Guarino M (2020) A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? *Journal of Cleaner Production* **262**, 121409.

Manning J, Power D and Cosby A (2021) Legal complexities of animal welfare in australia: Do on-animal sensors offer a future option? *Animals* **11**, 1–15.

Martiskainen P, Järvinen M, Skön JP, Tiirikainen J, Kolehmainen M and Mononen J (2009) Cow behaviour pattern recognition using a three-dimensional accelerometer and support vector machines. *Applied Animal Behaviour Science* **119**, 32–38.

Méndez-Ortíz FA, Sandoval-Castro CA, Vargas-Magaña JJ, Sarmiento-Franco L, Torres-Acosta JFJ and Ventura-Cordero J (2019) Impact of gastrointestinal parasitism on dry matter intake and live weight gain of lambs: A meta-analysis to estimate the metabolic cost of gastrointestinal nematodes. *Veterinary Parasitology* **265**, 1–6.

Merenda VR, Ruiz-Munoz J, Zare A and Chebel RC (2021) Predictive models to identify Holstein cows at risk of metritis and clinical cure and reproductive/productive failure following antimicrobial treatment. *Preventive Veterinary Medicine* **194**, 105431.

Michie C, Andonovic I, Davison C, Hamilton A, Tachtatzis C, Jonsson N, Duthie CA, Bowen J and Gilroy M (2020) The Internet of Things enhancing animal welfare and farm operational efficiency. *Journal of Dairy Research* **87**, 20–27.

Nasrollahi SM, Imani M and Zebeli Q (2016) A meta-analysis and meta-regression of the impact of particle size, level, source and preservation method of forages on chewing behavior and ruminal fermentation in dairy cows. *Animal Feed Science and Technology* **219**, 144–158.

O'Leary NW, Byrne DT, O'Connor AH and Shalloo L (2020) Invited review: Cattle lameness detection with accelerometers. *Journal of Dairy Science* **103**, 3895–3911.

Pan H, Zou Y and Gu M (2022) A spectrum estimation approach for accurate heartbeat detection using Doppler radar based on combination of FTPR and TWV. *Eurasip Journal on Advances in Signal Processing* **2022**.

Paudyal S, Maunsell FP, Richeson JT, Risco CA, Donovan DA, & Pinedo, PJ (2018) Rumination time and monitoring of health disorders during early lactation. *Animal: an international journal of animal bioscience*, **12**, 1484–1492.

Pavlovic D, Czerkawski M, Davison C, Marko O, Michie C, Atkinson R, Crnojevic V, Andonovic I, Rajovic V, Kvascev G *et al.* (2022) Behavioural Classification of Cattle Using Neck-Mounted Accelerometer-Equipped Collars. *Sensors* **22**, 1–18.

Pereira GM, Heins BJ, O'Brien B, McDonagh A, Lidauer L and Kickinger F (2020) Validation of an ear tag–based accelerometer system for detecting grazing behavior of dairy cows. *Journal of Dairy Science* **103**, 3529–3544.

Perez MM, Cabrera EM and Giordano JO (2023) Effect of targeted clinical examination based on alerts from automated health monitoring systems on herd health and performance of lactating dairy cows. *Journal of Dairy Science*.

Phillipson AT (1939) The movements of the pouches of the stomach of sheep. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences* **29**, 395–415.

Plaizier JC, Mulligan FJ, Neville EW, Guan LL, Steele MA and Penner GB (2022) Invited review: Effect of subacute ruminal acidosis on gut health of dairy cows. *Journal of Dairy Science* **105**, 7141–7160.

Plaza MA, Arruebo MP, Sopena J, Bonafonte JI and Murillo MD (1996) Myoelectrical activity of the gastrointestinal tract of sheep analysed by computer. *Research in Veterinary Science* **60**, 55–60.

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reid CSW and Titchen DA (1959) Activity of the partially exteriorized reticulum and rumen in conscious sheep. *J.Physiol.* 14–15.

Reiter S, Sattlecker G, Lidauer L, Kickinger F, Öhlschuster M, Auer W, Schweinzer V, Klein-Jöbstl D, Drillich M and Iwersen M (2018) Evaluation of an ear-tag-based accelerometer for monitoring rumination in dairy cows. *Journal of Dairy Science* **101**, 3398–3411.

Riaboff L, Shalloo L, Smeaton AF, Couvreur S, Madouasse A and Keane MT (2022) Predicting livestock behaviour using accelerometers: A systematic review of processing techniques for ruminant behaviour prediction from raw accelerometer data. *Computers and Electronics in Agriculture* **192**, 106610.

Rial C, Laplacette A, Caixeta L, Florentino C, Peña-Mosca F and Giordano JO (2023) Metritis and clinical mastitis events in lactating dairy cows were associated with altered patterns of rumination, physical activity, and lying behavior monitored by an ear-attached sensor. *Journal of Dairy Science* **106**, 9345–9365.

Rodriguez Z, Kolar QK, Krogstad KC, Swartz TH, Yoon I, Bradford BJ and Ruegg PL (2023) Evaluation of reticuloruminal temperature for the prediction of clinical mastitis in dairy cows challenged with Streptococcus uberis. *Journal of Dairy Science* **106**, 1360–1369.

Rojo-Gimeno C, van der Voort M, Niemi JK, Lauwers L, Kristensen AR and Wauters E (2019) Assessment of the value of information of precision livestock farming: A conceptual framework. *NJAS - Wageningen Journal of Life Sciences* **90–91**, 100311.

Rombach M, Münger A, Niederhauser J, Südekum KH and Schori F (2018) Evaluation and validation of an automatic jaw movement recorder (RumiWatch) for ingestive and rumination behaviors of dairy cows during grazing and supplementation. *Journal of Dairy Science* **101**, 2463–2475.

Rotz CA and Thoma G (2017) Assessing carbon footprints of dairy production systems. In

Large Dairy Herd Management, pp 19–32. American Dairy Science Association.

Ruckebusch Y (1974) Sleep deprivation in cattle. *Brain Research* **78**, 495–499.

Ruckebusch Y (1993) Motility of gastrointestinal tract. In *The Ruminant Animal: Digestive Physiology and Nutrition*, pp 64–107. Ed D Church. Waveland Press.

Ruckebusch Y and Tomov T (1973) The sequential contractions of the rumen associated with eructation in sheep. *The Journal of Physiology* **235**, 447–458.

Ruckebusch and Y. (1983) Pharmacology of reticulo-ruminal motor function. *Journal of Veterinary Pharmacology and Therapeutics* **6**, 245–272.

Scheurwater J, Hostens M, Nielen M, Heesterbeek H, Schot A, van Hoeij R and Aardema H (2021) Pressure measurement in the reticulum to detect different behaviors of healthy cows. *PLoS ONE* **16**, 1–13.

Schirmann K, von Keyserlingk MAG, Weary DM, Veira DM and Heuwieser W (2009) Validation of a system for monitoring rumination in dairy cows. *Journal of Dairy Science* **92**, 6052–6055.

Schirmann K, Chapinal N, Weary DM, Heuwieser W and von Keyserlingk MAG (2012) Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science* **95**, 3212–3217.

Schweinzer V, Gusterer E, Kanz P, Krieger S, Süss D, Lidauer L, Berger A, Kickinger F, Öhlschuster M, Auer W *et al.* (2019) Evaluation of an ear-attached accelerometer for detecting estrus events in indoor housed dairy cows. *Theriogenology* **130**, 19–25.

Sellers AF and Stevens CE (1966) Motor functions of the ruminant forestomach. *Physiological Reviews* **46**, 634–661.

Semiadi G, Muir PD, Barry TN, Crush JR and Hodgson J (1993) Grazing patterns of sambar deer (Cervus unicolor) and red deer (Cervus elaphus) in captivity. *New Zealand Journal of Agricultural Research* **36**, 253–260.

Sjaastad O., Sand O and Hove K (2016) The Digestive System. In *Physiology of Domestic Animals*, Third, pp 629–724. Ed SV Press. Oslo.

Song X, van der Tol PPJ, Groot Koerkamp PWG and Bokkers EAM (2019) Hot topic: Automated assessment of reticulo-ruminal motility in dairy cows using 3-dimensional vision. *Journal of Dairy Science* **102**, 9076–9081.

Stafford KJ, Reid CSW, Barry TN and Barry TN (1993) Rumino-reticular motility in red deer (census elaphus) fed chaffed lucerne hay during winter and summer. *New Zealand Journal of Agricultural Research* **36**, 465–473.

Stangaferro ML, Wijma R, Caixeta LS, Al-Abri MA and Giordano JO (2016a) Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. *Journal of Dairy Science* **99**, 7422–7433.

Stangaferro ML, Wijma R, Caixeta LS, Al-Abri MA and Giordano JO (2016b) Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metabolic and digestive disorders. *Journal of Dairy Science* **99**, 7395–7410.

Stangaferro ML, Wijma R, Caixeta LS, Al-Abri MA and Giordano JO (2016c) Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part II. Mastitis. *Journal of Dairy Science* **99**, 7411–7421.

Stevenson JS (2022) Late-gestation ear-surface temperatures and subsequent postpartum health, activity, milk yield, and reproductive performance of dairy cows. *Theriogenology* **181**, 170–179.

Stygar AH, Gómez Y, Berteselli G V., Dalla Costa E, Canali E, Niemi JK, Llonch P and Pastell M (2021) A Systematic Review on Commercially Available and Validated Sensor Technologies for Welfare Assessment of Dairy Cattle. *Frontiers in Veterinary Science* **8**, 1–15.

Tafaj M, Zebeli Q, Baes C, Steingass H and Drochner W (2007) A meta-analysis examining effects of particle size of total mixed rations on intake, rumen digestion and milk production in high-yielding dairy cows in early lactation. *Animal Feed Science and Technology* **138**, 137–161.

Ternman E, Hänninen L, Pastell M, Agenäs S and Nielsen PP (2012) Sleep in dairy cows recorded with a non-invasive EEG technique. *Applied Animal Behaviour Science* **140**, 25–32.

Tucker CB, Jensen MB, de Passillé AM, Hänninen L and Rushen J (2021) Invited review: Lying time and the welfare of dairy cows. *Journal of Dairy Science* **104**, 20–46.

Vázquez-Diosdado JA, Miguel-Pacheco GG, Plant B, Dottorini T, Green M and Kaler J (2019) Developing and evaluating threshold-based algorithms to detect drinking behavior in dairy cows using reticulorumen temperature. *Journal of Dairy Science* **102**, 10471–10482.

Werner J, Umstatter C, Leso L, Kennedy E, Geoghegan A, Shalloo L, Schick M and O'Brien B (2019) Evaluation and application potential of an accelerometer-based collar device for measuring grazing behavior of dairy cows. *Animal* **13**, 2070–2079.

White RR, Hall MB, Firkins JL and Kononoff PJ (2017) Physically adjusted neutral detergent fiber system for lactating dairy cow rations. I: Deriving equations that identify factors that influence effectiveness of fiber. *Journal of Dairy Science* **100**, 9551–9568.

Wierzbicka M, Domino M, Zabielski R and Gajewski Z (2021) Long-term recording of reticulo-rumen myoelectrical activity in sheep by a telemetry method. *Animals* **11**, 1–14.

Williams LR, Moore ST, Bishop-Hurley GJ and Swain DL (2020) A sensor-based solution to monitor grazing cattle drinking behaviour and water intake. *Computers and Electronics in Agriculture* **168**, 105141.

Wittek T, Locher LF, Alkaassem A, & Constable, PD (2009) Effect of surgical correction of left displaced abomasum by means of omentopexy via right flank laparotomy or two-step laparoscopy-guided abomasopexy on postoperative abomasal emptying rate in lactating dairy cows. *Journal of the American Veterinary Medical Association*, **234**, 652–657.