

## Research Article

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

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## 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  $40.4 \pm 4.7$ ,  $40.1 \pm 4.0$  and  $38.4 \pm 3.3$  s. Bland–Altman 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.

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 intercontraction 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.

## Materials and methods

### 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  $623.5 \pm 31.15$  kg (mean  $\pm$  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 straw-bedded pen ( $\sim 100$  m<sup>2</sup>) throughout the study. The total feed fence and water trough lengths were 9 and 1.1 m, 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) (detailed in online Supplementary Table S1).

Two devices each equipped with a tri-axial 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 3.5 MHz 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 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.

### 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.

### 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  $ICI = 600 \text{ s}/\text{number of contractions}$ . Statistical analyses were performed using R (R core Team, 2020), using the ‘ggplot’, ‘tidyverse’, ‘lubridate’, and ‘mgcv’

packages. Distributions were checked. Pearson's correlation coefficients were calculated and Bland–Altman 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 'mgcv', with up to 24 knots, to define the effects of hour of day (diurnal pattern).

## Results

**Table 1** 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  $40.4 \pm 4.7$  and  $40.1 \pm 4.0$ , 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.

The Bland–Altman plot shows the indwelling reticuloruminal bolus agreement with the ultrasound examination (**Fig. 1**). 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. S1 (ICI values for individual cows measured during the clinical examination) and **Fig. S2** (the same parameters comparing AM and PM time periods).

**Figure 2** 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** 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 (online Supplementary Fig. S3).

**Figure 3** shows the diurnal pattern of reticuloruminal motility measured by the indwelling boluses for each individual cow. A common diurnal pattern can be seen for all the animals. The cyclic GAM is summarised in **Table 2**: 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 4** 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)  $\sim 10$  s and ICI  $\sim 50$ – $60$  s and a minor peak at CD  $\sim 8$  s and ICI  $\sim 40$ – $45$  s. Cow 994 showed the same major peak, but the minor peak was less evident.

## Discussion

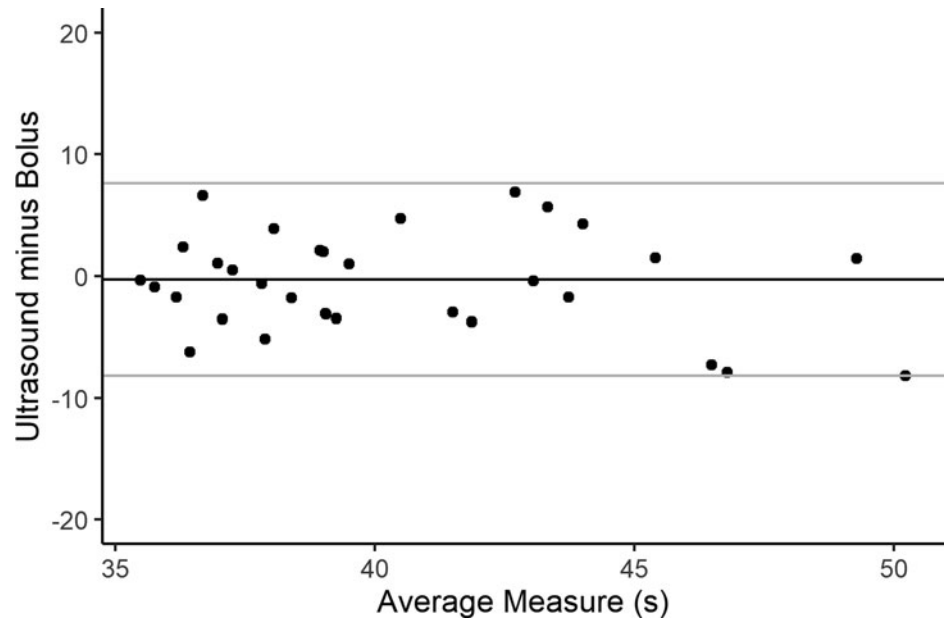
We used an indwelling bolus and a neck collar, both equipped with a triaxial accelerometer, to characterise the diurnal pattern

**Table 1.** Summary of the descriptive statistic

Variable	Number of observations	Minimum	1st Quartile	Median	Mean	SD	3rd Quartile	Max	Mean (h/d*)
AUSCICI <sup>a</sup>	36	33.3	37.5	37.5	38.4	$\pm 3.3$	40	46	NA.
Auscultation inter contraction interval (s)									
USICI <sup>a</sup>	35	33.3	37.5	40.1	40.1	$\pm 4.0$	42.9	50	NA
Ultrasound inter contraction interval (s)									
BICI <sup>a</sup>	582	24	32	40	40.4	$\pm 4.7$	46	47.7	NA
Bolus inter contraction interval (s)									
BICI <sup>b</sup>	99 907	24	38	44	46.9	$\pm 13.5$	40.4	236	NA
Bolus inter contraction interval (s)									
BCD <sup>b</sup>	99 907	6	7.5	9	9.3	$\pm 1.9$	11	15	NA
Bolus contraction duration (s)									
BRT <sup>b</sup>	1296	0	9.5	26.2	23.9	$\pm 16.1$	35.9	60	9.6
Bolus rumination time (min/h)									
CRT <sup>b</sup>	1296	6	6	22.5	21.4	$\pm 15.4$	33	60	8.6
Collar rumination time (min/h)									
CET <sup>b</sup>	1296	0	0	15.8	22	$\pm 21.9$	40.5	61.5	8.8
Collar eating time (min/h)									

<sup>a</sup>Data were collected during the 10 min of clinical examination.

<sup>b</sup>Data were collected during the 14 d study period.

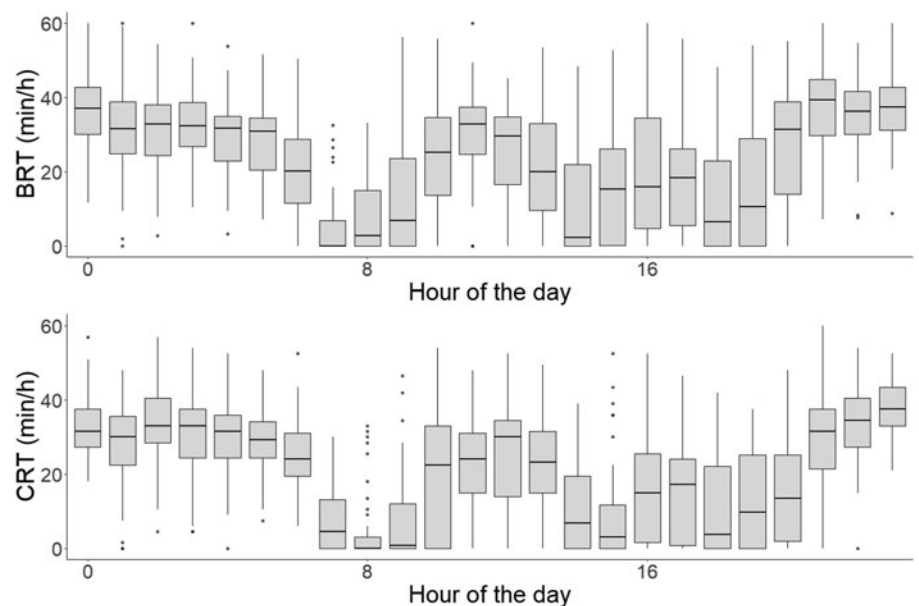


**Fig. 1.** Bland–Altman plot agreement for indwelling boluses (smaXtec, Austria) and ultrasonography examination in the 10 min observational period. The *x*-axis shows mean values for bolus and ultrasound ICI, and the *y*-axis shows the difference in values (seconds) between the USICI and the BICI. The black horizontal line in the middle indicates the mean, and the horizontal grey lines represent the 95% CI.

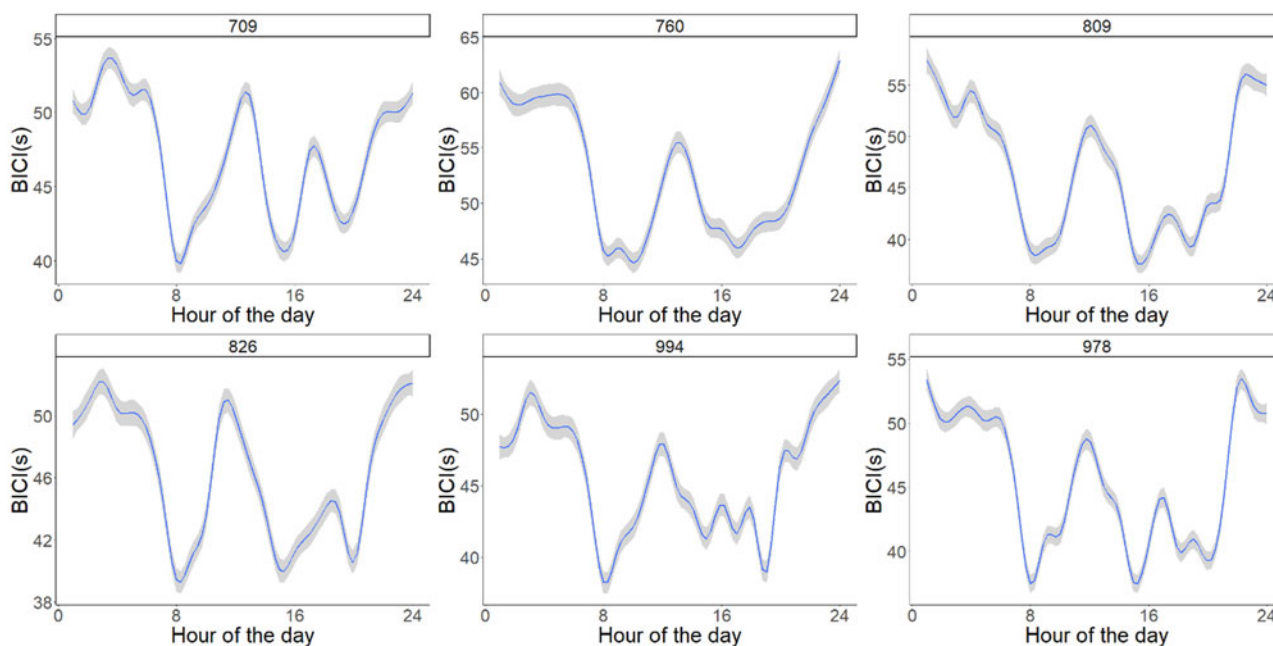
of time spent ruminating, and the bolus was also used to measure RRRCR. The RRRCR data provided by the indwelling boluses were consistent with the ultrasonographic examination, and there was an excellent correspondence between the diurnal patterns of the proportion of time ruminating from the indwelling boluses and the neck collars.

The first objective of our study was to determine whether the indwelling bolus provided observations on RRRCR that were consistent with those obtained from clinical examination using auscultation and ultrasound. Although there is no recognised gold standard to measure RRRCR, 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 ( $2.5 \pm 0.32$ ), 4.1–6.7 s ( $5.3 \pm 1.02$ ), and 2.2–7.5 s ( $5.0 \pm 0.83$ ), respectively. The number of contractions in 9 min of examination for the first, second reticular, and rumen atrium contractions were 6–17 ( $11.0 \pm 2.12$ ), 6–17 ( $11.0 \pm 2.12$ ), 6–15 ( $10.7 \pm 2.10$ ), 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  $\sim 47$  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  $\sim 51$  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



**Fig. 2.** Boxplots showing the diurnal pattern of the BRT (upper plot) and the CRT (lower plot), measured in min/h over 24 h. The boxes' bottoms and tops represent the first and third quartiles; the heavy black horizontal lines represent the median. The whiskers indicate 1.5 times the interquartile range, and the black dots are the outliers.



**Fig. 3.** Cyclic generalised additive model (GAM) of the diurnal reticularuminal inter-contraction interval measured by the indwelling boluses (y-axis) by hour of day (x-axis), for each cow for data from the entire study period.

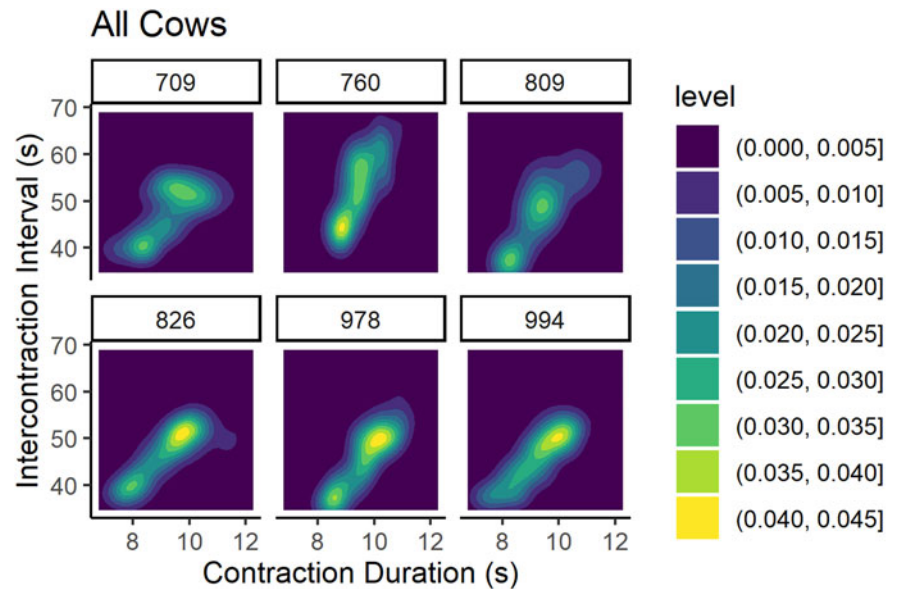
standardise the clinical observation period in our study, frequency spectrum resolution inevitably introduces potential for error in our clinical estimates of ICI. We attempted to estimate the period between contractions during a finite window of observation, commencing with the identification of a reticular 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

**Table 2.** 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 $\sim$ 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	$3.7 \times 10^{-5}$
Cow 826	-0.903	0.136	-6.643	$3.1 \times 10^{-11}$
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	22 625.4	$<2 \times 10^{-16}$	





**Fig. 4.** Density plot of data from the entire study period showing the bolus inter contraction interval (ICI, s) on y-axis and bolus contraction duration (BCD, s) in the x-axis. Each panel shows one of the cows in the study. For each of the cows there seem to be two points of higher density of observations, likely consistent with two distinct types of contraction: one with a relatively shorter contraction duration and period, and one with longer duration and period.

studied (Stangaferro *et al.*, 2016a, 2016b, 2016c; 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 reticulorumen 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  $\sim$ 10 s and ICI  $\sim$ 50–60 s and a minor peak at CD  $\sim$ 8 s and ICI  $\sim$ 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 cranio-caudal 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  $\sim$ 39, and the CD  $\sim$ 7.3 s; during resting, the ICI was  $\sim$ 49.5 s, the CD was  $\sim$ 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  $\sim$ 8 s, and ICI was  $\sim$ 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 RRRCR 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 RRRCR using a commercial indwelling bolus, supporting the use of these devices to assess reticulorumen motility. Further investigation of the relationship between ICI and CD in health and disease should enable early diagnosis of disease conditions using this technology.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029923000134>.

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