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DOI link to the version of record on the publisher’s site

Charlton, G.L., Churches, J., Bleach, E.C.L. and Thorup, V.M. (2024) ‘Behaviour of Cows with Johne’s Disease (Paratuberculosis)’, *Ruminants* 4(4), pp.533-542.

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Article

Behaviour of Cows with Johne's Disease (Paratuberculosis)

Gemma L. Charlton [1,*], Jeanette Churches [1], Emma C. L. Bleach [2] and Vivi M. Thorup [3]

[1] Animal Behaviour and Welfare Research Group, Animal Science Research Centre, Harper Adams University, Edgmond, Shropshire TF10 8NB, UK; jechurches00@gmail.com
[2] The Dairy Cow, Heifer and Calf Group, Animal Science Research Centre, Harper Adams University, Edgmond, Shropshire TF10 8NB, UK; ebleach@harper-adams.ac.uk
[3] Department of Animal and Veterinary Sciences, Aarhus University, 8830 Tjele, Denmark; vivim.thorup@anivet.au.dk
* Correspondence: gcharlton@harper-adams.ac.uk

Simple Summary: Johne's disease (JD) has detrimental effects on production and health and significantly reduces animal welfare. A recent study during peak lactation revealed that cows with JD (JD5) reduced their lying time compared to cows without JD (JD0). However, their step count was unaffected by JD, suggesting that the cows were standing idle, ruminating, or possibly standing eating. The objective of our study was to compare feeding behaviours and activity of JD5 cows to JD0 cows. The results support previous findings, with JD5 cows having lower lying times but no difference in step count. In addition, around week 8 of lactation, JD5 cows spent over 1 h/d longer ruminating, but there was no difference in eating times. Although it is still unclear what the cows were doing during these periods of reduced lying, this study does confirm behavioural difference between JD5 and JD0. Future research to further explore the difference in behaviour between JD5 and JD0 cows is recommended.

Abstract: Johne's disease (JD) significantly reduces the welfare of cattle worldwide. As changes in lying and feeding behaviours are considered important tools for assessing health and early detection of diseases, the aim of this study was to compare lying and feeding behaviours of JD-positive (JD5) and JD-negative (JD0) cows around peak lactation. The cows were fitted with an accelerometer-based sensor to record step counts and lying behaviour. They were also fitted with a pressure-based halter from approximately 56 d post-partum to collect feeding and rumination data. Every 3 months, the cows were milk sampled to test for naturally occurring JD using an ELISA. JD5 cows [n = 14 (two positive results in any four consecutive ELISAs)] were matched to JD0 cows [n = 14 (consecutive negative ELISAs)] based on lactation stage, parity, age, and milk yield. Of the 28 cows, 9 JD5 and 9 JD0 cows provided sensor data for analysis. JD5 cows spent 1.7 h/d less lying compared to JD0 cows. No differences in time spent eating were found; however, JD5 cows spent 1.1 h/d longer ruminating and produced 80 more feed boluses/d than JD0 cows around week 8 of lactation. The reason JD5 and JD0 cows behave differently around peak lactation is unclear and therefore warrants further investigation focusing on behaviour, milk yield, and feed intake among cows with JD.

Keywords: Johne's disease; paratuberculosis; MAP; lying behaviour; feeding behaviour; rumination

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Citation: Charlton, G.L.; Churches, J.; Bleach, E.C.L.; Thorup, V.M. Behaviour of Cows with Johne's Disease (Paratuberculosis). *Ruminants* **2024**, *4*, 533–542. https://doi.org/10.3390/ruminants4040037

Academic Editors: Leilson Rocha Bezerra and Phillip Lancaster

Received: 17 September 2024
Revised: 5 November 2024
Accepted: 6 November 2024
Published: 11 November 2024

1. Introduction

Johne's disease (JD, also known as paratuberculosis) is a chronic wasting disease in ruminants caused by *Mycobacterium avium* subspecies paratuberculosis (MAP; [1]). JD is a complex, global health issue, and it is considered endemic in countries with large dairy industries, including Australia, Canada, Denmark, the Netherlands, the United Kingdom, and the United States of America [2,3]. Annual global losses due to JD are estimated at USD 4B [4], with economic losses caused by decreased milk yield, fertility, and feed conversion efficiency, as well as increased culling rates, replacement costs, and disease susceptibility [1,5,6]. Johne's disease in dairy cattle has an environmental impact, increasing greenhouse gas emissions per unit of milk by up to 24% [7]. Additionally, associations have been made between MAP and Crohn's disease in humans [8].

Detection of JD is difficult, as MAP has a long incubation period of between two and seven years [9]; therefore, the majority of infected cattle are subclinically ill. Despite appearing healthy, subclinical cows can still shed bacteria in faeces, thus contaminating the environment and exposing other animals to MAP [10]. Primarily, clinical signs are loose faeces and gradual loss of body condition, which progresses to profuse scour, rapid weight loss, and lethargy in the terminal stage [10,11]. The delay in onset of these non-specific clinical signs makes JD difficult to diagnose [12].

Behavioural changes resulting from the disease may occur before clinical signs develop [13]. Hence, recording and interpreting animal behaviour can aid in the diagnosis of health issues [14]. For example, in the 2 weeks before calving, cows later diagnosed with metritis spent around 40 min/d less lying and had fewer lying bouts compared with healthy cows [15]. In addition, during the 3 d before metritis diagnosis, metritic cows ate approximately 1 kg/d less and consumed fewer meals compared with healthy cows. Furthermore, on the day that lame cows were diagnosed, they spent half as long ruminating compared to healthy cows [16]. Being able to diagnose the disease earlier could improve animal welfare and reduce economic losses [13]. Although there is no effective treatment for cattle with JD, identifying infected cows earlier would allow control measures to be more effectively implemented, thus helping to prevent further disease transmission [1,17].

A study by Charlton et al. [18] was the first to investigate the activity of JD-positive (at least two positive ELISA results; JD5) cows during the preclinical phase. The study found that during peak lactation (week 8), JD5 cows had reduced lying time, spending an average of two hours per day less lying than JD-negative (consecutive negative ELISA results; JD0) cows. However, there was no difference in the number of steps per day of JD5 and JD0 cows, indicating that cows spent more time standing still during this period of reduced lying. As no feeding behaviours were recorded by Charlton et al. [18], it is unclear what the cows were doing during this time.

As a gastrointestinal disease, JD causes malabsorption of nutrients. Consequently, JD5 cows may have spent more time standing and eating each day to meet the energy demands of peak lactation [19]. Therefore, the aim of this study was to determine whether JD5 dairy cows reduce their lying time and spend more time eating and ruminating around peak lactation compared to JD0 cows.

2. Materials and Methods

2.1. Animals and Management

Data collection was carried out in 2018, 2019, and 2021 at Harper Adams University, UK, using 28 lactating, multiparous (range: 2–9 lactations) Holstein Friesian cows. The cows were milked twice per day in a 40-point Westfalia internal rotary parlour, starting at 5:00 a.m. and 3:00 p.m. The cows were housed in freestall yards with others from the milking group of approximately 180 cows with 1.3 m × 2.5 m cubicles (approximately 110 cubicles per 100 cows) equipped with 3 cm deep latex mattresses with a rubber top-cover. The cubicles were bedded with fresh sawdust twice weekly. Passageways were scraped five times a day by automatic scrapers.

Fresh total mixed ration (dry matter (DM) 41.4%, metabolizable energy 12.1 MJ per kg DM, crude protein 16.9% of DM, neutral detergent fibre 35.6% of DM) was given at approximately 8:00 a.m. daily, which is sufficient for ad libitum intake, and it was pushed up to the feed barrier a minimum of five times a day by an automated feed pusher. The cows had ad libitum access to water from tipping water troughs. Ethical approval for the study was given by Harper Adams University Research Ethics Committee (protocol-5646-201712).

2.2. Selection of the Study Cows

Milk samples were collected from all lactating cows in the dairy herd and tested for naturally occurring JD every three months (National Milk Records (NMR; Chippenham, UK)) using the commercial milk Mycobacterium Paratuberculosis Antibody Screening ELISA (Idexx Laboratories Inc., Westbrook, ME, USA; [20]). According to NMR [21], when this ELISA is conducted on milk samples from individual cows, it has a sensitivity of 40–80% and specificity > 99%. Table 1 shows how the JD status of cows was classified based on these milk ELISA results. Cows who had repeated negative test results (minimum two tests) were classed as JD-negative (JD0), and cows who had two or more positive results in the previous four tests, at any time in their testing history, were classed as JD-positive (JD5). The cows' JD status was monitored throughout and after the end of the study to ensure it did not change. Additionally, their health was checked, and apart from half of them testing positive for JD, all cows were healthy upon selection and remained healthy. The JD5 cows were in the subclinical phase of JD throughout the study, exhibiting no apparent clinical signs. Prior to the start of the study, JD5 cows (n = 14) were paired with JD0 cows (n = 14) based on the stage of lactation, parity, age, and milk yield. The data from each pair were collected within a 4-week period to balance for the season and year. The number of JD5 cows was limited due to a JD control program on the research farm.

Table 1. Classification and definition of Johne's disease (JD) infection groups from National Milk Records (NMR), UK.

Risk Level	Classification	JD Infection Group	Definition
Low	Green	JD0	Repeat ELISA negative (minimum 2 tests)
Low	Green	JD1	ELISA negative but only one test
High	Amber	JD2	ELISA negative but 1 positive within 3 tests
High	Amber	JD3	ELISA negative but positive on previous test
High	Amber	JD4	ELISA positive, all previous tests negative
High	Red	JD5	Repeat ELISA positive (minimum 2 tests)

[21].

2.3. Behaviour Recordings

From after parturition and throughout the study, all cows wore an IceQube accelerometer-based sensor (Peacock Technology (formerly IceRobotics), Stirling, UK), attached using a Velcro strap on the back-left leg. IceQube sensors were previously validated for lying time, standing time, frequency of lying bouts, and step count in cubicle housing and straw yards [22]. The IceQubes use a 3-axis accelerometer to record the lying time, standing time, frequency of lying bouts, and step count with a granularity of 15 min (Table 2). These activity data were stored within the sensor before automatic downloading onto the CowAlert system (Peacock Technology (formerly IceRobotics), Stirling, UK) when the cow passed a reader in the entrance to the milking parlour twice daily. Daily sums were calculated for all variables (see Table 2). Due to missing data, accelerometer data were available for n = 9 pairs of cows (n = 9 JD0 cows and n = 9 JD5 cows).

From approximately 56 d post-partum (when the largest behavioural difference was previously observed [18]), the cows were each fitted with a RumiWatch halter (Itin and Hoch GmbH, Liestal, Switzerland) to collect seven consecutive days of data. The RumiWatch halter collects data via a pressure sensor and a triaxial accelerometer, whereby it measures feeding behaviour and rumination from jaw activity, which was previously validated [23]. The feeding-related activities analysed in the present study are described in Table 2. For this study, eating time with head up and eating time with head down ('Eat1 time' and 'Eat2 time', respectively) and chewing feed before swallowing with head up and chewing feed before swallowing with head down ('Eat1 chew' and 'Eat2 chew', respectively) were combined to generate the total eating time and the total number of eating chews, respectively. The halter was fitted so that the band around the lower jaw and the nose was 11–16 cm above the tip of the nose and it had 3–5 cm of movement space between it and the nose bridge [23]. Data were downloaded using the RumiWatch Manager software (Version 1.16; Itin and Hoch GmbH, Liestal, Switzerland) twice per week (at three- to four-day intervals). The first 48 h of recorded data were not analysed to allow the cows an adjustment period to the halter. RumiWatch data were available for n = 9 pairs of cows (n = 9 JD0 and n = 9 JD5 cows), with some data from the original dataset of 28 cows excluded due to device malfunctions and the analysis being limited to lactation weeks 8 and 9.

Table 2. Descriptions of behaviours recorded by the IceQube and RumiWatch sensors.

Behaviour	Description
IceQube variables	
Lying time	When the sensor is horizontal and the cow lies on the sternum or side.
Lying bout	The number of times the cow lies down.
Standing time	When the sensor is vertical and the body is supported by all four legs.
Step count	The number of times the cow lifts a foot and sets it down to walk or run to a new position.
RumiWatch variables	
Eating time	Ingesting and masticating food with head positioned down or up.
Eating chews	Opening and closing the jaw to masticate fresh food with head positioned down or up.
Bolus count	A regurgitated mass of cud, which is swallowed again after chewing, counted when a mass of cud is regurgitated.
Rumination time	Time spent regurgitating and rechewing boluses of food.
Rumination chews	Opening and closing the jaw to chew regurgitated food.
Chews per bolus	Chews between the regurgitation and swallowing of one bolus.

(Source: adapted from Peacock Technology, formerly IceRobotics, not dated; [23]).

2.4. Statistical Analysis

Weekly means per cow for weeks 8 (days in milk (DIM) 50–56) and 9 (DIM 57–63) of lactation were calculated from the daily sums for all variables measured by the RumiWatch halters and the IceQubes, and they were analysed separately through one-way ANOVA to enable comparison with a previous study [18].

Data were checked for normality. Step counts and lying bouts were log-transformed for normal distribution. The leg sensor variables were tested separately in the same non-linear mixed effects model using the lme function in R-package nlme (version 3.1-163). Longitudinal studies of loose-housed cows suggest that the lying time and the step count decreased to a nadir around 4–5 weeks post-partum and then increased [24–26]. Considering these indications of non-linear trends in the behaviour of cows during early lactation and the current study using frequent observations during DIM 1–70, cow behaviour was approximated with a polynomial function for DIM in model M1:

$$[\text{M1}]\ Y \sim \alpha + \text{JD-status} + (\beta + \text{JD-status}) \times \text{DIM} + (\gamma + \text{JD-status}) \times \text{DIM}^2 + \text{calving year} + \text{calving season} + \text{cow}$$

where JD-status (JD0; JD5), calving year (2018, 2019, 2021), and calving season (early = January to April; late = June to September) were fixed factors, DIM and DIM^2 were fixed, continuous effects, two-way interactions between JD-status and DIM and JD-status and DIM^2 were included as fixed effects, and the cow was a random effect. Temporal autocorrelations in covariance were accounted for. More specifically, the AR1 structure was used, which specifies that the correlations between the repeated measurements of each cow decrease with the time lag, and DIM was used to determine the correct order and define the time lag. Variance heterogeneity was accounted for by allowing different variance for each level of JD-status.

The effect of JD-status was tested using a χ^2 likelihood ratio test (LRT) comparing M1 with the model with the same polynomial coefficients for the two levels, that is, the reduced model M1, where JD-status (and interactions with it) were removed. When the polynomials differed significantly, the coefficients for each level of JD-status were reported. When the polynomials did not differ, the model was reduced, and the LS means (S.E.) reported for each level of the JD-status (JD0 or JD5) were averaged over the levels of the calving year (2018, 2019, or 2021) and the calving season (early or late).

Variance heterogeneity was tested by comparing models with and without heterogeneity components using an LRT after REML (restricted maximum likelihood) estimation. Significance was declared at $p \leq 0.05$.

3. Results

The comparison of variables for weeks 8 and 9 of lactation is provided in Table 3. During week 8, the time spent ruminating and the number of boluses regurgitated were significantly higher in JD5 cows compared with JD0 cows (Table 3). JD5 cows ruminated for 1.1 h/d longer and regurgitated 80 more boluses of feed/d compared to JD0 cows. There was no difference in the time spent eating or the other variables measured using the RumiWatch halters with respect to JD-status during week 8 or during week 9.

Table 3. Means (s.d.) of weekly averages of sensor variables of Johne's disease negative (JD0) and positive (JD5) cows during weeks 8 and 9 of lactation. IceQube sensors: 18 cows (n = 9 JD0 and n = 9 JD5) contributed 234 days of data, n = 35 weekly averages. RumiWatch sensors: 18 cows contributed 112 days, n = 30 weekly averages.

	Week 8			Week 9		
	JD0	**JD5**	***p* Value**	**JD0**	**JD5**	***p* Value**
			IceQube variables			
Lying Time, h/d	12.5 (2.73)	11.1 (1.08)	0.19	12.2 (2.64)	10.9 (1.37)	0.21
Lying Bouts, no/d	12.0 (4.04)	10.6 (4.31)	0.50	11.1 (3.92)	9.5 (2.99)	0.34
Standing Time, h/d	11.5 (2.73)	12.9 (1.08)	0.19	11.8 (2.64)	13.1 (1.37)	0.21
Step Count, no/d	778 (321)	1061 (534)	0.20	801 (378)	1192 (684)	0.15
			RumiWatch variables			
Eating Time, h/d	5.5 (0.67)	5.9 (0.78)	0.34	5.3 (0.96)	5.1 (0.52)	0.56
Eating Chews, no/d	21,998 (2374)	23,576 (4387)	0.32	21,239 (3489)	19,826 (4379)	0.50
Bolus, no/d	553 (46.1)	633 (93.0)	0.04	562 (55.0)	614 (124.1)	0.28
Rumination Time, h/d	9.1 (0.70)	10.2 (0.82)	0.02	9.2 (0.76)	9.8 (1.6)	0.38
Rumination Chews, no/d	35,744 (2758)	38,456 (3577)	0.13	35,441 (2481)	37,294 (7566)	0.50
Chews per Bolus, no/d	51.1 (4.48)	54.6 (4.53)	0.18	51.6 (5.24)	49.2 (8.02)	0.49

The mixed modelling showed that the lying time curves for JD0 (n = 9) and JD5 (n = 9) differed significantly (LRT = 14.9; df = 3; $p = 0.002$), that is, JD5 cows laid down less (−1.7 h/d) during days 1 to 70 compared to JD0 cows (Figure 1A). Equations (1) and (2) enabled us to plot the estimated curves for the lying time of JD0 cows and JD5 cows (see Figure 1A).

$$Y_{JD0} = 14.14 - 0.012 \times DIM - 0.0001 \times DIM^2 \text{ (h/d)} \quad (1)$$

$$Y_{JD5} = 12.36 - 0.078 \times DIM + 0.0009 \times DIM^2 \text{ (h/d)} \quad (2)$$

The JD0 and JD5 curves for Log(lying bouts) did not differ (LRT = 7.66; df = 3; $p = 0.054$); see Equations (3) and (4), with the estimated curves shown in Figure 1B. The back-transformed intercepts of the JD0 and JD5 curves were 12.3 and 10.6 lying bouts/d, respectively.

$$\text{Lying bouts}_{JD0} = 1.091 - 0.00005 \times DIM - 0.00002 \times DIM^2 \text{ (h/d)} \quad (3)$$

$$\text{Lying bouts}_{JD5} = 1.024 - 0.00223 \times DIM + 0.00003 \times DIM^2 \text{ (h/d)} \quad (4)$$

The curves of the two JD statuses did not differ significantly (LRT = 1.49; df = 3; p = 0.684) for log(Step count); thus, JD0 = 3.04 (0.071) and JD5 = 3.09 (0.054), which corresponds to 1096 and 1230 steps/d when back-transformed.

The variance was not heterogenous for any of the features (lying time, lying bouts, and step count), meaning the JD status did not cause more or less variation in these features.

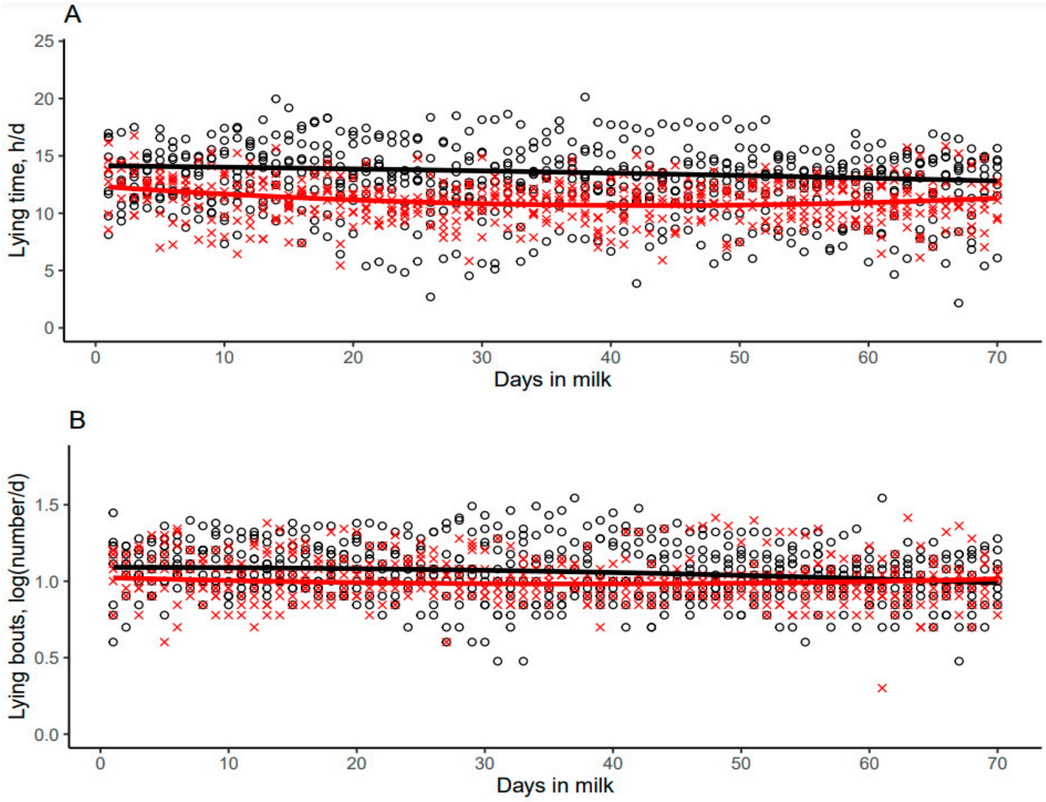

Figure 1. Lying behaviour during 1 to 70 days in milk in Johne's disease positive (JD5; n = 9) and negative (JD0; n = 9) cows under model M1. (**A**) Lying time (h/d). (**B**) Lying bouts (log (number/d)). JD0 estimate: black line; JD5 estimate: red line. Individual observations shown as black hollow circles (JD0) or red crosses (JD5).

4. Discussion

JD5 cows spent less time lying down than JD0 cows in early lactation, which confirms previous findings [18]. It was speculated by Charlton et al. [18] that the JD5 cows may have spent their increased standing time at the feed fence eating. The current study does not support this supposition, as eating times during week 8 and 9 of lactation were similar in JD5 and JD0 cows. However, during week 8 of lactation, in the present study, JD5 cows increased their rumination time and the number of boluses produced, so it is possible that the JD5 cows were standing and ruminating, although cows have been shown to more commonly ruminate while lying down [27].

Lying is a high-priority activity for dairy cattle, and lying time must be sufficient to promote milk production, welfare, and foetal development [28–30]. During the current study, the cows laid down for an average of 11.8 h/d. This is within the expected range of 9.5 to 12.9 h/d for lactating cows housed in freestalls [31], but lying times did vary, ranging from 6.9 to 14.9 h/d. A study using 3649 cows from 100 dairy herds also found a similarly wide variation in individual daily lying time, ranging from 6.3 to 17.9 h/d [32]. In the present study, there was a significant difference in the lying time curves of JD5 and JD0 cows, with JD5 cows lying on average 1.7 h/d less than JD0 cows. Similarly, during our previous study on the effects of JD on cow activity, JD5 cows spent up to 2 h/d less lying compared to JD0 cows [18]. The stocking rate of cubicles and at the feed fence can influence the lying time, the time spent standing idle, and the eating time [33,34]. In the current study, sufficient cubicles and feed space were provided to allow all cows to lie down and to eat simultaneously; therefore, the stocking rate should not have affected activity like lying times or feeding behaviours. However, paratuberculosis may cause gastrointestinal discomfort [9], which may have caused the JD5 cows to reduce their lying time and spend more time standing.

During weeks 8 and 9 of lactation, the average lying bout frequency was 10.8 times per day, which is within the expected range of 9 to 11 bouts per day [35,36]. Lying bout frequency curves did not differ between JD0 and JD5 cows. In comparison, Charlton et al. [18] recorded significant differences, with JD5 cows having 2.7 and 2.5 fewer lying bouts/d at weeks 8 and 9, respectively, of lactation, with this further decreasing to 3.6 fewer lying bouts/d at week 11. Other conditions affect lying bout frequency. Cows with mastitis have shown an increase in lying bouts, thought to be due to udder discomfort [37]. The frequency of lying bouts also increased in the 6 h prior to parturition [38,39], possibly due to pain and restlessness. Throughout the current study, apart from half of the cows having JD, they were all otherwise healthy, with no signs of pain, distress, or disease.

Similarly to previous findings [18], there was no difference in step count between JD0 and JD5 cows in the present study. Other diseases have caused cows to increase or decrease their step count. For example, cows suffering from mastitis take more steps compared to healthy cows [37], whereas lame cows decrease their stepping behaviour, possibly due to pain and discomfort caused by hoof and leg disorders [40–42]. Our results suggest that during periods of reduced lying, the cows were standing still. It remains unclear what they were doing during this time, which warrants further investigation. However, it is possible that the JD5 cows spent more time standing and ruminating. In support of this, cows with digital dermatitis reduced their lying time and spent more time ruminating while standing compared to healthy control cows [43].

Rumination is a vital part of the feeding process for ruminants, playing a key role in maintaining the optimum rumen pH, minimising the risk of rumen acidosis, enhancing fibre digestion, and breaking down particles for their movement from the rumen to the lower gastrointestinal tract [44]. In a review, a large variability in rumination time was reported, with mean rumination of 7.3 h/d, ranging from 3.9 to 10.2 h/d [45]. In the current study, average rumination times were at the higher end of this range at 9.2 and 10.0 h/d for JD0 and JD5 cows, respectively, during weeks 8 and 9 of lactation, possibly due to the chemical and physical composition of the diet [44].

During week 8 of lactation, JD5 cows spent 1.1 h/d longer ruminating and produced 80 more boluses per day compared to JD0 cows. This finding contrasts with the decrease in eating and rumination behaviours often seen in lactating dairy cows in response to disease or illness [46]. For example, lame cows reduced their rumination time and number of boluses up to 14 days before the onset of the clinical signs of lameness [16]. In addition, they reduced their eating time, drinking time, and eating and rumination chews. Fogsgaard et al. [47] found that cows with acute, clinical mastitis spent less time ruminating and eating following diagnosis. In addition, cows with subclinical ketosis have been found to reduce their time spent ruminating compared to cows with no recorded health problems [48]. Rumination time is considered an important tool for assessing dairy cow health and the early detection of diseases [49]; therefore, it may be a useful addition to a multifaceted diagnostic tool for JD.

The reason that JD5 cows produced more boluses and spent longer time ruminating is unclear. Animal variability, physical and chemical composition of the diet, and accuracy of measurement techniques have been discussed as reasons for variability in rumination time [44]. As the JD5 and JD0 cows were housed together and offered the same diet, and the same halters were used to collect data, neither the diet nor the measurement technique should have affected the rumination time in the present study. JD causes malfunction of the intestinal tract, particularly whilst nutrient demand is at its highest during peak lactation [19,50], and this may have affected rumination times. However, rumination is a complex phenomenon, and further investigation, including a much larger sample size, is required to understand the complexities of rumination and the effect of JD on rumination times.

Eating times and eating chews were similar for JD0 and JD5 cows at week 8 and week 9 of lactation. Unfortunately, information on eating behaviour was only available from 18 cows due to a limited number of JD5 cows in the herd and because of technological failures, resulting in missing data, which is a limitation of the current study. Research has shown large variability in eating times between cows fed TMR [44,45]; therefore, a greater sample size may have been required to find significant differences in eating times between JD0 and JD5 cows. Future research should aim to include a much larger sample size to further investigate eating behaviours and explore the DMI of dairy cows in the subclinical and clinical stages of JD. With more focus on sustainable dairy herds, it would be useful, alongside milk production, to determine the feed efficiency of JD cows, so that farmers can keep the most profitable cows in the herd and make strategic culling decisions based on lifetime cumulative costs and revenues [51] and to improve herd health. Despite the low sample size in the current study, the behaviour of cows with JD is an under-investigated research area, and the findings of this study and our previous study [18] confirm behavioural differences between JD5 and JD0 cows.

With dairy farmers increasingly using wearable sensors to constantly record activities, such as the lying times and step counts of cows [52], the present study suggests that there are opportunities for future research to further focus on the behaviour of cows with JD under differing management systems and environments. Knowing how infected individuals deviate from those that are healthy will help farmers identify subclinically ill cows and allow us to understand how they cope in these systems.

5. Conclusions

Cows with subclinical JD showed reduced lying time during early lactation, but their step count was not different to JD0 cows. There were no differences in eating times, but JD5 cows spent longer ruminating during week 8 of lactation. This study indicates that there are some differences in the feeding behaviours of JD0 and JD5 cows that warrant further investigation. Future research should also investigate the DMI and feed efficiency of cows with JD.

Author Contributions: Conceptualisation, G.L.C., V.M.T., and E.C.L.B.; methodology, G.L.C.; software, V.M.T. and G.L.C.; validation, G.L.C.; formal analysis, V.M.T.; investigation, G.L.C. and J.C.; resources, G.L.C.; data curation, V.M.T.; writing—original draft preparation, G.L.C.; writing—review and editing, G.L.C., V.M.T., E.C.L.B., and J.C.; visualisation, G.L.C.; supervision, G.L.C.; project administration, G.L.C.; funding acquisition, G.L.C. and V.M.T. All authors have read and agreed to the published version of the manuscript.

Funding: The RumiWatch halters were funded by Innovate UK.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Harper Adams University (protocol code 5646-201712 and date of approval 9 December 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: The authors thank Carrie Gauld and Sarah Williams at Harper Adams University for their assistance in collecting the data.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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