

Characterizing changes in activity and feeding behaviour of housed, lactating dairy cows during behavioural and silent oestrus

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1 **Characterizing changes in activity and feeding behaviour of housed, lactating**
2 **dairy cows during behavioural and silent oestrus**

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17 **Highlights**

- 18 • Activity was increased on the day of behavioural oestrus
19 • Resting time, lying bouts and feeding behaviours were reduced on the day of behavioural
20 oestrus
21 • Only feeding duration was reduced on the day of silent oestrus

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26 **Abstract**

27 The normal time budgets of dairy cows are influenced by oestrus, with cows spending less
28 time resting and eating but more time walking. Previous studies have shown that cows spend
29 approximately 21% less time feeding where the day of oestrus is assumed to be the day of
30 successful artificial insemination. The objective of the present study was to determine whether
31 the number of steps, lying time, lying bouts, dry matter intake (**DMI**), feeding duration and the
32 number of visits to feed were affected by behavioural and silent oestrus in lactating dairy cows.
33 Thirty Holstein Friesian cows were housed in a free-stall barn with 34 cubicles and were
34 continuously monitored by four video cameras. Milk samples were collected on Monday,
35 Wednesday and Friday afternoon and analysed for progesterone concentration by enzyme
36 immunoassay. Steps, lying time and lying bouts were measured using IceQubes (IceRobotics
37 Ltd., Edinburgh, UK) from three days before (3DB) to three days after (3DA) oestrus. Daily
38 feed intakes and feeding duration were recorded by a Roughage Intake Control (RIC) system
39 (Insentec B. V., Marknesse, Netherlands) over the same period.

40 Of the 40 *behavioural* oestrus events, standing behaviour was observed in 50% of events.
41 On the day of *behavioural* oestrus the number of steps were increased significantly ($P < 0.001$)
42 compared to 3DB and 3DA oestrus, whilst the percentage of lying time, lying bouts, DMI,
43 feeding duration and the number of visits to feed were reduced ($P < 0.001$) compared to 3DB
44 and 3DA oestrus. On the predicted day of *silent* oestrus, the duration of feeding was reduced
45 ($P < 0.03$) only when compared to one day before and one day after oestrus.

46 In conclusion, although the number of steps were increased, lying time, lying bouts, DM
47 intake and feeding duration were reduced by behavioural oestrus, and only feeding duration
48 was significantly lowered during silent oestrus. Technologies that facilitate the on-farm
49 measurement of feeding duration could potentially be used to help farmers detect silent
50 oestrus in their cattle.

51

52 **Keywords:** Dairy cows; Time budgets; Behavioural oestrus; Silent oestrus; Activity

53

54 **1. Introduction**

55 The normal time budget of a Holstein dairy cow fed a total mixed ration (**TMR**) and in free-
56 stall housing is 3 to 5 h/d eating, with an average 14 feeding bouts per day, 12 to 14 h/d lying
57 time, 2 to 3 h/d social interaction, 7 to 10 h/d rumination during both standing and lying time,
58 0.5 h/d drinking and 2.3 to 3.5 h/d spent outside of the yard for milking and other management
59 practices (Grant and Albright, 2000).

60 In mammals, oestrus is a behavioural sign that ensures that the female is ready to be mated
61 close to the time of ovulation. Mounting behaviour with standing to be mounted is the definitive
62 sign of oestrus (Roelofs *et al.*, 2010). However, over the past 30 to 50 years, the incidence of
63 mounting behaviour has decreased from 80% to 50% in dairy cows (Dobson *et al.*, 2008) and
64 over the last 50 years the duration of oestrus in dairy cattle has also declined from 18 to 8 h
65 (Dolecheck *et al.*, 2015). Oestrus is the period of maximum sexual activity, it has been shown
66 to range from 2-30 h (Hanzen, 2000). Standing oestrus is often defined as true oestrus, when
67 the cow makes no effort to escape when mounted by other cows and is defined as “the interval
68 between the first and last standing events” (Hurnik *et al.*, 1975). Other signs of oestrus include
69 mounting of other cows, increased activity and mucus discharge from the vulva (Sveberg *et al.*
70 *et al.*, 2011). While standing to be mounted is considered as the primary behavioural sign of
71 oestrus, other behaviours such as ano-genital sniffing, restlessness, bellowing, chin resting,
72 head mounting, and an attempt to mount are considered secondary signs (Gordon, 2011).

73 The cows' normal time budget can be influenced by oestrus (Yaniz *et al.*, 2006). During
74 oestrus, the activity of dairy cows increases about 2 to 4 times compared to non-oestrus cows
75 (Kiddy, 1977). In addition, during the period from 72 to 16 h before standing oestrus, dairy cow
76 activity increases linearly with further increases during the 16 h before standing oestrus (Arney
77 *et al.*, 1994). In dairy cows ovulation occurs from 8 to 30 h after the onset of increased activity
78 (Hocky *et al.*, 2010). With the availability of activity monitoring on commercial dairy farms,
79 restlessness has become an important indicator of oestrus (Diskin and Sreenan, 2000).

80 During oestrus, the time spent lying by dairy cows decreased as a result of increased
81 activity and restlessness (Jónsson *et al.*, 2011) driven by increased secretion of oestradiol

82 (Sumiyoshi *et al.*, 2014) from the developing ovulatory follicle (Allrich, 1994). According to
83 Dolecheck *et al.* (2015) oestrus-synchronised cows spent less time lying than non-oestrus
84 cows (10.19 vs 24.82 min/h, respectively) when IceQubes were used to monitor activity and
85 Reith *et al.* (2014) found that dairy cows drank 15.3% less water during oestrus. In a study
86 where the day of AI was assumed to be the day of oestrus (rather than observing for oestrus
87 behaviour), Halli *et al.* (2015) found that cows spent approximately 21% less time feeding on
88 the day of oestrus in comparison to other days of the oestrous cycle (2.82 vs. 3.54 h/d,
89 respectively), but it was unclear whether the cows were synchronised or naturally cycling. In
90 addition, Reith and Hoy (2012) showed that rumination was reduced on the day of oestrus
91 from 7.2 to 5.9 h/d.

92 However, 35 % of cows show no obvious behavioural signs of oestrus and are defined as
93 showing silent oestrus (Palmer *et al.*, 2010). This means that despite the use of oestrus
94 detection aids such as activity monitors, judging the correct time for AI in naturally cycling
95 cows is difficult. The present study was designed to investigate whether the activity and
96 feeding behaviour of lactating Holstein Friesian cows undergoing spontaneous oestrus cycle
97 is affected by behavioural and silent oestrus.

98 **2. Materials and methods**

99 The experiment was undertaken between June and August 2016 at the dairy unit of Harper
100 Adams University, Newport, Shropshire, TF10 8NB, UK. The Harper Adams University
101 Research Ethics Committee approved the research protocol.

102 **2.1. Experimental animal, housing and management**

103 Thirty Holstein-Friesian cows (parity 2.5 ± 1.1) with initial body weight of 637.0 ± 60.0 kg
104 and daily milk yield of 35.8 ± 1.8 kg/d, were used at Harper Adams University dairy unit. At the
105 start of the study the cows were 29 ± 6.3 days in milk and 2.9 ± 0.28 body condition score
106 (Scale 1-5; AHDB Dairy, 2014). The average locomotion score (Scale 1-5; as described by
107 Chapinal *et al.*, 2009) of the selected cows was 2.0 ± 0.58 . Cows were housed in a covered

108 yard with 34 cubicles (2.7 x 1.2 m, with 3 cm thick rubber mattresses) and two grooved
109 concrete passageways (6 x 50 m) giving approximately 10.8 m² area per cow. The cubicles
110 were bedded with sawdust three times per week. The passageways were scrapped by an
111 automatic scraper 4-5 times per day. Study cows were milked twice a day from approximately
112 05:00 and 16:30 through a 40-point internal rotary milking parlour (Wesfalia, GEA Milking
113 System, Germany). Milking took approximately 30-40 minutes for the group.

114 Cows were fed from 30 Roughage Intake Control (RIC) system bins and intake recorded
115 using an automated feed recording system (1.0 x 0.9 x 0.8 m; RIC, Insentec B. V. Marknesse,
116 the Netherlands). They were moved into the study area on 6th June 2016 and data were
117 collected until 19th August 2016. All the cows used in the study were trained to feed through
118 RIC bins over a one week period in order to ensure that each cow could access feed without
119 assistance. Approximately 65 kg (fresh weight) of a total mixed ration (TMR) (see Table 1)
120 was provided daily at approximately 08:30, sufficient for *ad libitum* availability. Refused feed
121 was removed three times per week on Monday, Wednesday and Friday morning at 08:00 and
122 the RIC bins were cleaned before fresh feed was allocated. Water was provided *ad libitum*
123 from three water troughs. Feed samples were collected directly from the RIC bins daily at
124 feeding time and immediately oven dried overnight at 105°C to constant weight (AOAC, 2012;
125 934.01) for determination of dry matter (DM). The nutrient content of the ration composed of
126 DM (39.5%), ME (11.8 MJ/kgDM), CP (17.6% DM) and NDF (36.4% DM).

127 **2.2. Data collection**

128 **2.2.1. Video recording of oestrus behaviours**

129 The cows were monitored to detect spontaneous behavioural oestrus using four video
130 cameras (Voltek, KT&C Co Ltd, Seoul, South Korea) for approximately 19.46 ± 1.7 h/d. The
131 four cameras were placed at about 5.25 m above the trial cubicles and passageways to give
132 a clear view of the area in which cows were housed. The cameras were connected to an
133 external hard drive video recorder (Sentient 960H, England, UK). Cows were clearly identified
134 by numbers from 1 to 30 on both sides of the cow and an individual combination of coloured

135 tape on each cow (Kerbrat and Disenhaus, 2004). Video recordings were retrospectively
136 reviewed to determine the time and intensity of oestrus. The scores of Van Eerdenburg *et al.*
137 (2002) (Table 2) were allocated and recorded each time a sign of oestrus was observed on
138 the video recording. The total number of points scored in a day indicated oestrus intensity.

139 **2.2.2. Cow's activity and feed intake**

140 To monitor cow activity, IceQubes (IceRobotics Ltd., Edinburgh, UK) were attached to the
141 back left leg of each cow using a Velcro hook and loop strap (Dolecheck *et al.*, 2015). The
142 IceQube is a 3-axis accelerometer which reports cow activity summarised in 15 minute blocks
143 (Dolecheck *et al.*, 2015). These generate data to show *the number of steps taken, lying time*
144 *and lying bouts* for each cow, every day. Daily TMR intake was recorded by the RIC system.
145 Dry matter intake was calculated as TMR intake (fresh weight; kg/d) x dry matter of TMR. Total
146 daily feeding duration and the number of feeding bouts were also recorded by the RIC system.

147 **2.3. Milk progesterone assay**

148 Oestrus periods were identified by measuring the concentration of progesterone in whole
149 milk. Milk samples (40 ml) were collected from each cow 3 times per week on Monday,
150 Wednesday and Friday afternoon. Immediately after sampling one preservative tablet (Broad
151 Spectrum Microtabs II, Advanced Instrument, INC. USA; containing 8 mg Bronopol and 0.30
152 mg Natamycin) was added to each milk sample. Sample pots were inverted to mix until the
153 tablet was dissolved. The samples were stored in a refrigerator at 4°C until the progesterone
154 assay which was completed within one week of collection. Milk samples were brought to room
155 temperature and mixed well before analysis using an enzyme immunoassay (Ridgeway
156 Science Ltd., Rodmore Mill Farm, Alvington, Gloucestershire, UK). A cow was considered in
157 oestrus when milk progesterone concentrations were <3 ng/ml for two to three days before a
158 period when progesterone rose to >5 ng/ml for at least 5 days (Isobe *et al.*, 2004).

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161 **2.4. Definition of behavioural and silent oestrus**

162 Each oestrus identified by the progesterone profile was classified as a behavioural or silent
163 oestrus. A cow was defined to be in *behavioural oestrus* when the sum of the points scored
164 for oestrus behaviour observed by the video recording exceeded 100 (Van Eerdenburg *et al.*,
165 2002). A cow was considered to be in *silent oestrus* when the cow did not display any
166 behavioural signs of oestrus or the oestrus score was < 100 points at or around the day of
167 oestrus as defined by her milk progesterone profile (Van Eerdenburg *et al.*, 2002).

168 **2.5. Duration of oestrus**

169 The duration of oestrus recorded by the camera was defined as the interval between the
170 time that cows showed the first signs of oestrus and the time that the last signs of oestrus
171 were observed. Oestrus duration based on accelerometer data was defined as an increase in
172 walking activity and the number of steps taken increased to > 80% above the mean number
173 for the preceding three days followed by a decrease to < 80% in the following two days. The
174 periods between the two thresholds was considered as the duration of oestrus (Lopez-Gatius,
175 *et al.*, 2008).

176 **2.6. Data-set construction**

177 Data from 61 oestrus events were collected during the study period. The six parameters
178 analysed were *the number of steps taken by cows each day, time spent lying (h/d), number of*
179 *lying bouts per day, DM intake (kg/d), feeding duration (h/d) and number of visits to feed per*
180 *day*. Prior to statistical analysis, the data for all parameters were summarized to one value per
181 day using Microsoft Excel. A day was defined as the period from midnight to midnight. The
182 day of oestrus was defined as day (**0**) and compared with three days before (-3, -2 and -1;
183 3DB) and three days after (+1, +2 and +3; 3DA). Fresh TMR intakes (kg/d) intakes were
184 converted into DM intake (kg DM/d) based on the DM content of the TMR (39.5%).

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187 **2.7. Statistical analyses**

188 Statistical analyses were performed using the Genstat statistical software package
189 (Genstat V 17th edition, VSN international Ltd, UK). The datasets were analysed by repeated
190 measures ANOVA to compare between groups (*behavioural* and *silent oestrus*) days before
191 and after oestrus and the group x day interaction. Factorial one way ANOVA was used to
192 compare *behavioural* and *silent* oestrus. Differences were reported as significant at $P < 0.05$
193 and trends were reported when P was between < 0.1 and > 0.05 . Linear regression analysis
194 was used to determine the relationship between the number of steps taken per day and dry
195 matter intake (kg DM/d) on the day of behavioural oestrus.

196 **3. Results**

197 **3.1. The duration of oestrus and scores of behavioural activity**

198 Using the milk progesterone profiles, 61 spontaneous oestrus events were detected for all
199 cows during the study period. Of the 61 oestrus events detected, 40 were defined as
200 *behavioural* (65.5%) and 21 defined as *silent* (34.5%) oestrus. The percentage of *silent*
201 oestrus at the first, second and third oestrus post-partum were 44.8%, 27.3% and 20.0%
202 respectively in the present study. The percentage of cows standing to be mounted during
203 *behavioural* oestrus was 50%.

204 The average duration of *behavioural* oestrus (determined from video recordings) was $9.1 \pm$
205 3.1 h and the duration of oestrus based on the number of steps recorded by IceQubes was
206 12.9 ± 2.5 h. The number of points scored during *behavioural* oestrus determined from the
207 camera recordings were between 225 and 2921 points. However, during *silent* oestrus the
208 number of points scored were between 0 and 32 points.

209 **3.2. Oestrus activity**

210 On the day of *behavioural* oestrus the number of the steps (2095 ± 217 steps; mean \pm
211 SEM) were higher ($F_{6,354} = 32.2$, $P < 0.001$) compared to 3DB (849 ± 60 steps) and 3DA (971
212 ± 61 steps) while on the day of *silent* oestrus the number of steps (984 ± 73.5 steps) were not
213 significantly different in comparison to 3DB (891 ± 63 steps) and 3DA (849 ± 50 steps). From

214 factorial one way ANOVA, cows took significantly more steps during *behavioural* oestrus ($F_{1,39}$
215 = 13.2, $P < 0.001$) compared to *silent* oestrus. There was a significant interaction ($F_{6,354} = 5.6$,
216 $P < 0.001$) between oestrus expression and day of oestrus on the number of steps taken
217 (Figure 1, A). In addition, there was a significant ($F_{1,39} = 31.9$, $P < 0.001$) positive correlation
218 between the number of points scored and the number of steps taken ($y = 0.348x + 486$; $P <$
219 0.001 ; $r^2 = 0.32$) during *behavioural* oestrus.

220 Lying time and the number of lying bouts (7.1 ± 0.3 h/d and 9.1 ± 0.5 bouts, respectively)
221 were reduced ($F_{6,354} = 17.2$, $P < 0.001$) on the day of *behavioural* oestrus in comparison to
222 3DB (10.0 ± 0.3 h/d and 13.0 ± 0.7 bouts, respectively) and 3DA (10.1 ± 0.3 h/d and 12.7 ± 0.8
223 bouts, respectively). However, lying times (9.3 ± 0.5 h/d) and the number of lying bouts (13.0
224 ± 1.1) bouts were not significantly affected by the day of *silent* oestrus compared to 3DB (10.0
225 ± 0.4 h/d and 14.0 ± 1.2 bouts, respectively) and 3DA (10.4 ± 0.4 h/d and 13.7 ± 1.3 bouts,
226 respectively). Furthermore, from a factorial one way (ANOVA), lying times were lower ($F_{1,39} =$
227 17.2 , $P < 0.001$) on the day of *behavioural* oestrus compared to *silent* oestrus and the number
228 of lying bouts were also lower ($F_{1,39} = 17.2$, $P < 0.001$) on the day of *behavioural* oestrus
229 compared to *silent* oestrus. With regard to lying time, there was a significant oestrus x day
230 interaction ($F_{6,354} = 5.6$, $P < 0.001$) with lying time significantly reduced during *behavioural*
231 oestrus but not *silent* oestrus (Figure 1, B). Similarly, the number of lying bouts was reduced
232 during *behavioural* oestrus but not during *silent* oestrus as well as there being an interaction
233 between oestrus expression and day ($F_{6,354} = 3.3$, $P < 0.006$) (Figure 1, C).

234 3.3. Feeding behaviour

235 Dry matter intakes were significantly ($F_{6,354} = 12.0$, $P < 0.001$) lower on the day of
236 *behavioural* oestrus (19.8 ± 0.41 kg/d) in comparison to 3DB (22.4 ± 0.5 kg/d) and 3DA (22.6
237 ± 0.5 kg/d). There was a significant ($F_{1,39} = 31.9$, $P < 0.001$) negative correlation between the
238 number of steps taken and DMI ($y = -0.0014x + 22.46$; $P < 0.001$; $r^2 = 0.46$) during *behavioural*
239 oestrus. However, DMI was not significantly lower on the day of *silent* oestrus compared to
240 other days. There was also no interaction between oestrous expression and day (Table 3).

241 The occurrence of *behavioural* oestrus significantly ($F_{6,354} = 9.2$, $P < 0.001$) reduced the
242 mean duration of feeding (2.4 ± 0.09 h/d) in comparison to 3DB (3.4 ± 0.17 h/d) and 3DA (3.2
243 ± 0.12 h/d). Duration of feeding (2.9 ± 0.15 h/d) was also significantly ($P < 0.03$) reduced
244 during *silent* oestrus when compared to one day before (3.4 ± 0.2 h/d) and one day after (3.6
245 ± 0.2 h/d) the predicted day of oestrus. There was a tendency for an interaction between
246 oestrous expression and day ($F_{6,354} = 2.1$, $P = 0.06$) effect on feeding duration (Table 3).

247 The mean number of visits to the RIC feed bin during *behavioural* oestrus was less (25.3
248 ± 1.26 visits/d; $F_{6,354} = 9.5$, $P < 0.01$) compared with 3DB and 3DA oestrus. However, there
249 were no significant differences between the day of *silent* oestrus (29.0 ± 1.71 visits/d) in
250 comparison to other days. There was also no significant interaction between oestrus
251 expression and day with regard to the number of visits to feed. Analysing the number of visits
252 to feed with regard to oestrous expression, there was also no significant difference ($F_{1,59} =$
253 1.0 , $P = 0.318$) between *behavioural* and *silent* oestrus (Table 3).

254 **4. Discussion**

255 **4.1. Oestrus duration and observed oestrus activity**

256 Previously, the duration of standing oestrus in dairy cows has been considered to be 18 h
257 (Valenza *et al.*, 2012). The average duration of oestrus measured using the video camera in
258 the present study was 9.1 ± 3.1 h. However this was 2 hours longer than the duration reported
259 by Sveberg *et al.* (2011) of (7.1 ± 1.4 h) in oestrus detected by video recording of 22 Holstein-
260 Friesian cows housed on an outdoor wood chip-pad. Based on the number of steps recorded
261 by the IceQubes, the duration of oestrus in the present study was (12.9 ± 2.5 h) very similar
262 to that seen in the study of Roelofs *et al.* (2005) which found that the duration of oestrus
263 detected by pedometer lasted for 12.3 h, while a study by Silper *et al.* (2015), reported longer
264 oestrus in 12 month old Holstein heifers (14.3 ± 4.1 h) using neck mounted accelerometers.
265 However, the duration of oestrous activity in the present study was 3.2 h shorter than that
266 reported by Valenza *et al.* (2012) of 16.1 (± 4.7 h) also using an activity monitoring system.
267 The present study found that the duration of oestrus determined by activity monitor was 3 h

268 longer than that detected by observation. The difference between the duration of oestrus
269 activity in high yielding dairy cows may be due to the disconnection of secondary signs of
270 oestrus behaviour detected by activity monitors (restlessness) and standing oestrus (Valenza
271 *et al.* (2012). Our finding is supported by a reported increase in activity, detected by
272 pedometers in dairy cows, 1 to 3 h before the onset starting of standing oestrus (Sveberg *et*
273 *al.*, 2011).

274 In the present study, standing behaviour was observed in 50% of those cows expressing
275 behavioural oestrus. Similarly, Van Eerdenburg *et al.* (2002) detected 50% of standing
276 oestrus events in Holstein Frisian cows. However, Kerbrat and Disenhaus (2004) observed
277 standing events by video camera in 32% of Holstein cows housed in a loose housing system
278 with a concrete floor. The results of the present study indicate that there is great variability
279 between cows in the total points scored with between 225 and 2921 points during *behavioural*
280 oestrus and the number of steps taken during oestrus (754 to 6008 steps). The results of the
281 current study agree with those reported by Van Eerdenburg *et al.* (1996) who continuously
282 monitored cows and another study conducted on Holstein cows by Kerbrat and Disenhaus
283 (2004) who reported the total number of behavioural signs (rather than points score) which
284 ranged from 27 to 239 signs. As expected, the oestrus scores of the present study were
285 higher than the scores (approximately 50 -1000 points) reported by Van Eerdenburg *et al.*
286 (2000) who observed cows during two time periods of about 30 min in the morning at 5:00
287 before milking and 30 min in the afternoon at 17:00.

288 In dairy cows, oestrus often takes place without clear changes in behaviour (Kyle *et al.*,
289 1992). Indeed this was the case in 44.8% of first post-partum oestruses observed in the
290 present study. Low expression of oestrus behaviour at the first oestrus post-partum in
291 lactating dairy cows is thought to be an effect of high concentrations of oestradiol from foetal
292 origin during late gestation, which induces refractoriness of the hypothalamus to oestradiol
293 (Kyle *et al.*, 1992). Other studies suggested this may also be caused by lower frequency of
294 LH pulses as a result of negative energy balance in early lactation (Lucy, 2001) which results

295 in lower oestradiol synthesis (Butler, 2000; Isobe *et al.*, 2004) by the pre-ovulatory follicle and
296 decreased the sensitivity of the hypothalamus to oestradiol which leads to a high incidence
297 of *silent* oestrus.

298 **4.2. Oestrus activity detected by activity monitor**

299 Overall, 65.5% of the spontaneous oestruses identified in the present study were
300 associated with behavioural signs detected by video recording and the percentage of oestrus
301 detected by accelerometer was 52.5%. This was within the range 51 to 87% found by Roelofs
302 *et al.* (2005) using pedometers for oestrus detection. The results of the present study also
303 agree with the previously reported 52% detection rate in cubicle housed Holstein-Friesian
304 cows studied by Palmer *et al.* (2010). At-Taras and Spahr (2001) detected approximately
305 54% of oestrus by visual observation. Conversely, this finding was lower than the 70% of
306 oestrus events detected recorded by Fricke *et al.* (2014) in oestrus-synchronized Holstein
307 cows using an activity monitor attached to the neck (Heatime, SCR Engineer Ltd, Netanya,
308 Israel). This high detection rate may be related to the high number of cows in oestrus at the
309 same time (Gilmore *et al.*, 2011).

310 Restlessness is one of the most important secondary indicators of oestrus in cattle (Firk
311 *et al.*, 2002). In the current study, on the day of behavioural oestrus, the number of steps was
312 increased by 146.8%, while during silent oestrus, step count was only 10% higher. Similarly,
313 Sakaguchi *et al.* (2007) recorded a 100% increase in the number of steps on the day of
314 behavioural oestrus using radiotelemetric pedometers on grazing dairy Holstein heifers in
315 Japan. Using pedometers, ultrasound and visual observation, Roelofs *et al.* (2005) recorded
316 a 5.5 fold increase in the number of steps taken on the day of visually observed oestrus.
317 Environmental conditions, the type of housing and management conditions may affect the
318 extent of walking activity (Lopez-Gatius *et al.*, 2005; Yaniz *et al.*, 2006).

319 Alongside the increase in activity, on the day of *behavioural* oestrus in the present study,
320 cows spent significantly less time lying down (32.2%) and had 28.3% fewer lying bouts during
321 spontaneous oestrous events. On the day of behavioural oestrus, continuously observed

322 Friesian cows housed in cubicles were also found to spend less time lying down
323 (approximately 5 h/d) and more time standing than non-oestrus cows (Esslemont and Bryant,
324 1976).

325 A recent study at the University of Kentucky conducted by Dolecheck *et al.* (2015) using
326 oestrus-synchronised Holstein cows, found a 50% decrease in lying time and also a reduction
327 in lying bouts (56.0%) during oestrus. The greater reduction in lying behaviour may be
328 because oestrus synchronization meant there are more cows in oestrus at the same time
329 (Hurnik *et al.*, 1975) resulting in greater restlessness and activity on the day of behavioural
330 oestrus (Roelofs *et al.*, 2005; Jónsson *et al.*, 2011).

331 **4.3. Feeding behaviour**

332 In the present study, cows consumed approximately 22 kg DMI/d during a normal days,
333 similar to other published studies of early lactation cows: e.g. Dado and Allen (1994) reported
334 average DMI/d of 22.8 kg during a normal day. However, on the day of *behavioural* oestrus,
335 the increase in activity observed was associated with a 12% reduction in DMI. Furthermore,
336 both feeding duration and the number of visits to feed per day were lower on the day of
337 *behavioural* oestrus compare to 3DB and 3DA. These data suggest that increased activity at
338 oestrus diverts cows from their normal time budget with more steps replacing both feeding
339 and resting time (Walker *et al.*, 2008). This is exacerbated in more active cows which had a
340 greater reduction in DMI demonstrated by the negative correlation between the number of
341 steps taken and DMI during the day of behavioural oestrus. Other studies of Holstein-Friesian
342 cows have shown reduced DMI on the day of AI (14.6%; Reith *et al.*, 2014, 10.3%; Halli *et*
343 *al.*, 2015). Cows spent a similar amount of time feeding (2.8 h/d) but had many more visits to
344 the feed troughs (46.2 visits/d) than in the present study (Halli *et al.*, 2015). This may be
345 because Halli *et al.* (2015) only determined feeding behaviours in relation to AI rather than
346 behavioural oestrus.

347 As far as we are aware, the present study is the first to report feeding behaviour during
348 *silent* oestrus in dairy cows. Interestingly, while behavioural measures were not changed

349 during the predicted time of *silent* oestrus in the current study, DMI and number of visits to
350 the RIC bins were numerically lower in comparison to 3DB and 3DA oestrus. In addition
351 feeding duration was significantly reduced compared to one day before and one day after
352 oestrus. This finding indicates that cows deemed in *silent* oestrus may show subtle changes
353 to their behavioural repertoire that are not apparent using commercial oestrus detection
354 regimes. Alternatively, oestradiol has been shown to suppress feed intake (Ingvarlsen and
355 Andersen, 2000) and an increase in oestradiol concentration at silent oestrus may be
356 sufficient to reduce feed intake but not sufficiently adequate to increase oestrus activity. On
357 the day of oestrus, the higher physical activity and restlessness in dairy cows may replace
358 feeding behaviour (DMI, feeding duration and number of visits to feed) (Hurnik *et al.*, 1975;
359 Kiddy, 1977). In the present study there was a negative relationship between the number of
360 steps and DMI. In comparison to non-oestrus cows, during oestrus, cows spent more time
361 walking and consequently less time resting and eating (Hurnik *et al.*, 1975). Conversely,
362 Lukas *et al.* (2008) found that cows consumed more feed on the day of oestrus, while De
363 Silva *et al.* (1981) reported no change in feed intake on the day oestrus.

364 **5. Conclusion**

365 During the day of behavioural oestrus, high yielding dairy cows in cubicle housing spend
366 more time walking, less time lying down and a reduced number of lying bouts, but none of
367 these parameters were affected during silent oestrus. In addition, on the day of behavioural
368 oestrus, DM intake, feeding duration and number of visits to feed were reduced. On the day
369 of silent oestrus, only feeding duration was reduced. Technologies that facilitate the on-farm
370 measurement of feeding duration could potentially be used to help farmers detect silent
371 oestrus in their cattle. Where behavioural oestrus is expressed there is considerable variation
372 in the extent of activity, but the reasons for this remain to be elucidated. It remains to be
373 determined why these differences are seen but one factor worthy of investigation maybe
374 circulating oestradiol concentrations.

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378 farm staff and technicians (Carrie Gauld and Sarah Williams) for their assistance.

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514 **Table 1.** Dietary composition of the trial ration.

Ingredient	g/kg DM	kg DM/hd
Maize silage	342.2	7.2
Lucerne	161.60	3.4
Blend	200.57	4.22
Soda wheat	113.12	2.38
Sweet starch	73.19	1.54
Soya hulls	53.23	1.12
Spey syrup	26.62	0.56
Megalac	7.13	0.15
Butterfat extra	7.13	0.15
Dairy minerals	7.13	0.15
Salt	3.33	0.07
Acid buff	3.80	0.08
Saccharomyces cerevisiae	0.95	0.02
Total	1000	21.04

515 (Profeed Nutrition Consultancy, UK, 2016)

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527 **Table 2.** Scoring scale for observed signs of oestrous.

Oestrous signs	Score
Flehmen	3
Mucous discharge from vulva	3
Cow restlessness	5
Sniffing the vulva of another cow	10
Mounting but not standing	10
Resting the chin on the back of another cow	15
Mounting or attempt to mount other cows	35
Mounting or attempt to mount head side other cows	45
Standing heat	100

528 Van Eerdenburg *et al.*, 2002.

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540 **Table 3.** Means of dry matter intake (gkDM/d), feeding duration (h/d) and the number of
 541 visiting to feed/d, 3 days before, on the day of oestrus (0) and 3 days after oestrus, during
 542 behavioural (n = 40) and silent (n = 21) oestrus.

Feeding behaviour	Oe EX	Time/Day							SED	P-value		
		-3	-2	-1	0	1+	2+	3+		Oe Ex	Days	Oe Ex vs. days
Dry matter intake kg/d	B	22.5	22.1	22.5	19.8	22.5	22.5	22.7	0.906	0.314	<0.001	0.371
	S	21.9	21.8	21.6	20.5	21.8	21.8	22.0				
Feeding duration h/d	B	3.5	3.4	3.3	2.4	3.2	3.2	3.3	0.180	0.306	<0.001	0.06
	S	3.1	3.4	3.4	2.9	3.6	3.4	3.4				
Number of visits to feed/d	B	29.2	28.8	27.2	25.3	27.8	28.3	28.3	3.754	0.318	<0.01	0.588
	S	29.6	29.9	29	27.1	30.5	30.6	30.8				

543 Oe Ex = Oestrus Expression, B = Behavioural oestrus and S = Silent oestrus, 0 = day of
 544 oestrus, SED = standard errors of differences

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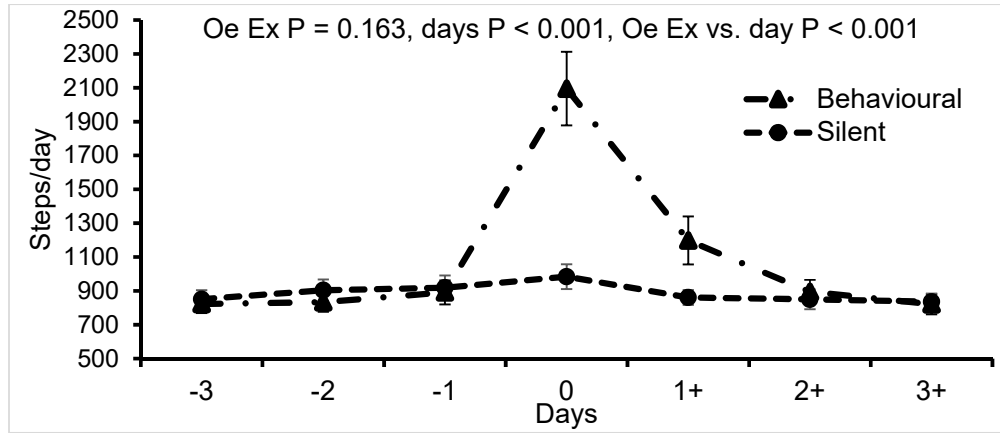
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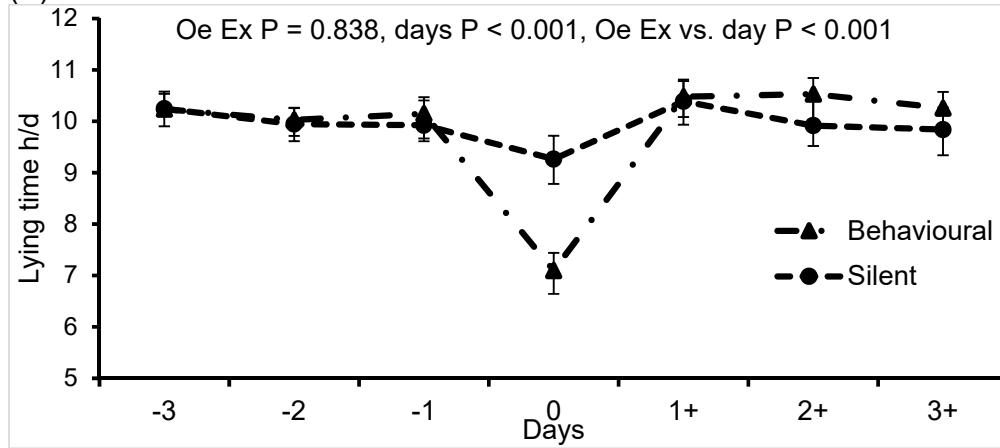


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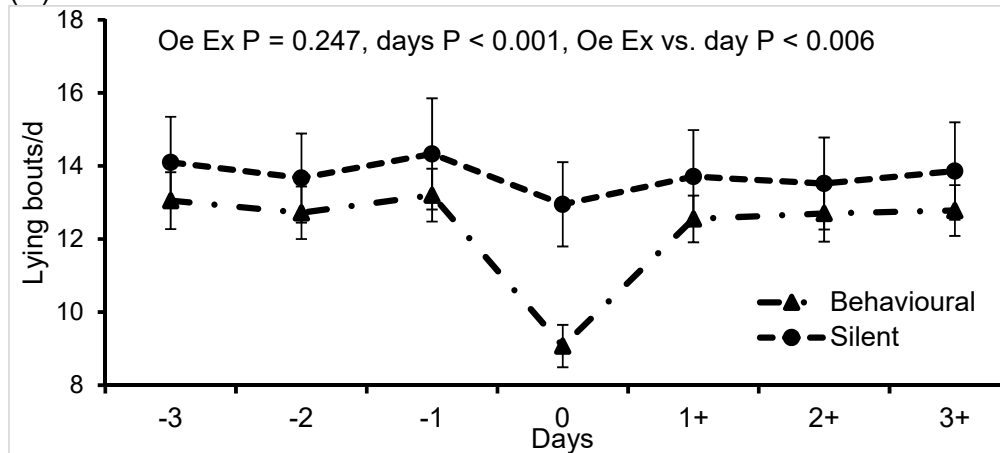
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(C)



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560 **Figure 1.** Effect of oestrus on number of steps (A), lying time (B) and number of lying bouts

561 (C), 3 days before, on day of oestrus (0) and 3 days after and during silent (n=21) and

562 behavioural (n=40) oestrus. Oe Ex = Oestrus Expression, 0=day of oestrus.