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Published in:
Applied Animal Behaviour Science

DOI:
[10.1016/j.applanim.2016.10.017](https://doi.org/10.1016/j.applanim.2016.10.017)

First published: 07/11/2016

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):

Kok, A., van Hoeij, R.J., Tolkamp, B.J., Haskell, M.J., van Kneegsel, A.T.M., de Boer, I.J.M., & Bokkers, E.A.M. (2016). Behavioural adaptation to a short or no dry period with associated management in dairy cows. *Applied Animal Behaviour Science*, 186, 7 - 15. Advance online publication. <https://doi.org/10.1016/j.applanim.2016.10.017>

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1 **Behavioural adaptation to a short or no dry period and associated management in dairy**
2 **cows**

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12

13 **ABSTRACT**

14 From calving, dairy cows are typically milked for about a year, and subsequently managed to have a
15 non-lactating or ‘dry period’ (DP) before next calving. However, the DP may reduce cow welfare
16 because of many DP-related changes in management and a severe negative energy balance after
17 calving. Shortening or omitting the DP may have beneficial effects on cow welfare through fewer
18 changes in management before calving, and a lower milk yield after calving. Our objective was to
19 assess the effects of a short DP (30 days) and no DP on feeding, lying, and number of steps of dairy
20 cows in late gestation and early lactation. Feeding behaviour was recorded by computerized feeders
21 for 122 periods (42 with a short DP and 80 with no DP) from week -6 to week 7 relative to calving.
22 Steps and lying behaviour of 81 of these cows (28 with a short DP and 53 with no DP) were recorded
23 with accelerometers in week -4 and in week 4 relative to calving only. Effects of DP treatment and
24 parity on behaviour were analysed with mixed models. Before calving, cows with a short DP were fed
25 a DP ration, and moved to a dry cow group. During this time, cows with a short DP spent more time
26 lying (13.7 vs. 12.6 h per day; $P = 0.01$) and feeding (240 vs. 209 min per day; $P < 0.01$), and stepped
27 less (663 vs. 1130 steps per day; $P < 0.01$) than cows with no DP. After calving, all cows were fed the
28 same lactation ration and were housed in the same herd. Cows with a short DP, however, had a lower
29 feed intake (35.7 vs 39.1 kg per day; $P < 0.01$), and spent less time lying (10.7 vs. 11.6 h per day; $P =$
30 0.03) after calving than cows with no DP. Milk yield was negatively correlated with daily lying time
31 ($r: -0.22$; $P < 0.05$), but was not correlated with daily feeding time. Also, less time was spent on both
32 lying and feeding after calving than before calving. These results indicate that lying time was not
33 constrained by feeding time. Lying time was positively correlated with energy balance ($r: 0.28$; $P <$
34 0.01). No DP, in comparison with a short DP and the associated changes in management, reduced
35 lying time and increased the number of steps in late gestation, and resulted in a higher feed intake and
36 longer lying time in early lactation.

37 **Keywords:** rest period, lying, feeding, cattle, transition period, sensor data

38 **1. INTRODUCTION**

39 The lactation cycle of dairy cows starts with calving. From calving, cows are typically milked for
40 about a year, and subsequently managed to have a non-lactating or 'dry period' (DP) of 6 to 8 weeks
41 before next calving. The DP allows for treatment of intramammary infections (Robert et al., 2006),
42 facilitates the renewal of udder cells (Capuco et al., 1997), and maximises milk yield in the next
43 lactation (Kuhn et al., 2005; van Knegsel et al., 2013). The DP is generally considered a rest period
44 for the cow that allows for reduced metabolic and physical activity in the last two months of
45 pregnancy.

46 Whether a DP is beneficial for dairy cow welfare has been questioned (Zobel et al., 2015). Good
47 welfare has been defined as feeling well, functioning well, and living a natural life (Fraser et al.,
48 1997). Planned cessation of lactation, as well as being unnatural, was shown to increase udder
49 pressure and stress (as measured by faecal glucocorticoid metabolites) at the start of the DP (Tucker et
50 al., 2009; Bertulat et al., 2013). In addition, cows need to adapt to a new social environment and to
51 dietary changes at the start and end of the DP, because they are typically moved to a non-lactating
52 group and fed a dry cow diet (von Keyserlingk et al., 2008; Martens et al., 2012; Santschi and
53 Lefebvre, 2014). After the DP, a higher milk yield is associated with a more severe negative energy
54 balance (Rastani et al., 2005; van Knegsel et al., 2014). Such a negative energy balance is associated
55 with impaired fertility and reduced metabolic health (Butler, 2003; Chen et al., 2015a; b), and may
56 last until 3 months into lactation after a conventional DP (Rastani et al., 2005; van Knegsel et al.,
57 2014). As a consequence of the prolonged lipolysis to meet energy needs, and possibly conflicting
58 motivations such as hunger, inappetence, and the desire to rest or ruminate, a cow may be in a
59 negative affective state during this period (Roche et al., 2009).

60 Behavioural adaptation may not interfere with welfare as long as it is within the limits of the adaptive
61 capacity of the animal (Korte et al., 2007). Behaviour of cows is affected by external factors (such as
62 housing) and internal factors (such as behavioural needs). The behaviour patterns that are expressed
63 are the result of these internal and external factors. Behaviour patterns can be assessed by examining

64 the time budget and the temporal distribution of behaviours (Winter and Hillerton, 1995). Much of the
65 time budget of dairy cattle is made up of lying, feeding, ruminating, and – in lactating cows – being
66 milked (Gomez and Cook, 2010; Norring et al., 2012). The daily duration of these activities depends
67 on factors such as housing, access to pasture, milking facilities, lameness, and stage of lactation
68 (Krohn et al., 1992; Huzzey et al., 2006; Fregonesi et al., 2007; Gomez and Cook, 2010). In addition
69 to changes in feeding time, cows were found to increase feeding rate when given limited access to
70 resources (Munksgaard et al., 2005) and when lame (González et al., 2008). To understand such
71 changes in short-term feeding behaviour, it is informative to cluster visits to the feeder into distinct
72 feeding bouts (meals) (Yeates et al., 2001; Tolkamp et al., 2002). Cow welfare may be compromised
73 when cows cannot adapt their behaviour to the circumstances, or if short-term behaviour patterns
74 result in a long-term reduction of welfare. Increased standing time, for example, is observed in early
75 lactation (Fregonesi and Leaver, 2001; Munksgaard et al., 2005), but (on hard surfaces) is a risk factor
76 for lameness (Cook and Nordlund, 2009).

77 Shortening or omitting the DP may have beneficial effects on cow welfare (Zobel et al., 2015). Both
78 strategies improve the energy balance after calving, through a reduced milk yield and equal or better
79 feed intake after calving (Rastani et al., 2005; van Knegsel et al., 2014). Moreover, milk yield before
80 dry-off is lower for a short DP than for a standard DP (Pezeshki et al., 2007), because milk yield
81 decreases towards the end of lactation. A lower milk yield before dry-off reduces udder pressure and
82 stress in the DP (Bertulat et al., 2013), and reduces the risk of intramammary infections after calving
83 (Rajala-Schultz et al., 2005). Cows with no DP can be kept in the herd, without regrouping and
84 dietary changes.

85 It is unclear how dairy cows adapt behaviourally to a DP, and how the absence of a DP affects their
86 time budget. Our objective, therefore, was to assess the effects of a short DP and no DP and
87 associated management on feeding, lying, and number of steps of dairy cows in late gestation and
88 early lactation. To assess possible reasons for changes in behaviour, we also studied associations
89 between behaviour, milk yield, and energy balance in early lactation.

90 **2. MATERIAL AND METHODS**

91 *2.1 Experimental design, animals, and housing*

92 The Institutional Animal Care and Use Committee of Wageningen University approved the
93 experimental protocol in compliance with Dutch law on Animal Experimentation (protocol number
94 2014125). The experiment was conducted at the Dairy Campus research farm (Lelystad, the
95 Netherlands) using 125 Holstein-Friesian cows between January 2014 and July 2015. The study was
96 initially designed to analyse the effect of DP length and dietary energy source on energy balance and
97 metabolic health; sample size was based on a power analysis for these variables. Cows were included
98 in the experiment at an average rate of 3 cows per week, based on the availability of cows in late
99 gestation. Inclusion criteria were an expected calving interval shorter than 490 days, a milk yield of
100 >16 kg and no clinical or subclinical mastitis (a cell count > 250.000 cells/ml) at 90 days before
101 expected calving. For practical reasons, six cows were used twice in the experiment, resulting in data
102 for 131 periods around calving (60 periods of cows in parity 1 before calving and 71 periods of cows
103 in parity > 1 before calving).

104 Treatment groups were balanced for parity (1 or > 1 before calving), expected calving date, and milk
105 production in the previous lactation. This was done by distributing clusters of 6 similar cows
106 randomly over no DP (n=87), or a short DP of 30 days (n=44), using a random number generator.
107 Twice as many cows were assigned to the no DP treatment because of an additional contrast in
108 concentrate allowance (further details will be given below).

109 Cows entered the experiment on Mondays, 44 ± 3 days before the expected calving date and were
110 kept in the study until 305 days in milk. All cows were housed in the same freestall barn with a
111 concrete slatted floor, and stalls fitted with rubber mattresses covered with sawdust. Lactating and dry
112 cows were kept in separate groups. The stocking density in both groups was maintained at one cow
113 per cubicle and two cows per feeding bin throughout the experiment, with a space allowance of 7 m²
114 per cow.

115 The drying-off protocol for cows with a short DP consisted of an abrupt transition to the DP ration at
116 day 7 before dry-off and an abrupt transition to milking once daily at day 4 before dry-off. Cows were
117 dried off (i.e. milked for the last time) on Mondays on 30 ± 3 days before the expected calving date.

118 At dry-off no antibiotics were used. Dry cows were weighed in the milking parlour on Tuesdays.

119 Lactating cows were milked and weighed in the milking parlour twice daily at about 06.00 h and
120 17.00 h.

121

122 ***2.2 Feed composition and provision***

123 During the DP, cows received a DP ration (estimated net energy (NE): 5.4 MJ per kg DM) that
124 consisted of grass silage, maize silage, wheat straw, and rapeseed meal in a ratio of 48:19:25:8 (DM
125 basis), and vitamins and minerals (for more detail see Van Hoeij et al., under review). Cows with no
126 DP received a lactation ration (estimated NE: 6.4 MJ per kg DM) that consisted of grass silage, maize
127 silage, wheat straw, soybean meal, and sugar beet pulp in a ratio of 45:35:2:8:10 (DM basis), and
128 vitamins and minerals. After calving, all cows received this lactation ration up to 49 days in milk
129 (DIM).

130 Basal rations were provided in roughage intake control (RIC) feeders (Insentec, Marknesse, the
131 Netherlands). One RIC feeder was available per two cows. Rations were mixed once daily before 10.00
132 h and fed twice daily around 10.00 h and 17.00 h. The RIC feeders could not be accessed by the cows
133 when feeders were filled and from 23.45 h to 0.00 h when data records were saved. Cows had free
134 access to water, that was provided in valve trough drinkers placed in between feeding bins and quick
135 drainage troughs of 150L at opposite sides of the barn. Because cow density was kept constant,
136 lactating cows had access to 3 or 4 troughs and dry cows had access to 1 or 2 troughs depending on
137 group size.

138 Concentrate was provided separately from the basal ration, and the concentrate allowance differed
139 between treatment groups. Cows with a short DP were fed a standard amount of concentrate for their
140 expected milk yield (Short DP STD). The expected daily milk yield in the 14 weeks after calving was
141 40.4 kg fat-and-protein-corrected milk (FPCM) for cows with a short DP and 35.4 kg FPCM for cows
142 with no DP, compared with 43.3 kg FPCM for cows with a standard DP (Van Knegsel et al., 2014).

143 Cows with no DP were assigned either to the same concentrate level as cows with a short DP (No DP
144 STD), or to a lower concentrate level that matched their expected milk yield (No DP LOW), .

145 All cows received concentrate (869 g DM per kg; estimated NE: 7.4 MJ per kg DM) at a level of 1 kg
146 per day from -10 ± 3 days before the expected calving date. The concentrate allowance increased

147 stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up to 8.5 kg per day at 28 DIM for the short
148 DP STD and no DP STD treatments, and stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up
149 to 6.7 kg per day at 22 DIM for the no DP LOW treatment. Concentrate was provided by two
150 computerized feeders located in the freestall (Manus VC5, DeLaval, Steenwijk, the Netherlands). The
151 individual daily allowance of concentrate was available in equal portions (minimum portion size: 0.4 kg)
152 over six 4-h periods, and the actual quantity dispensed (kg per day) was recorded. Uncollected
153 concentrate portions in one timeslot were added to the portion in the next timeslot. Additionally,
154 lactating cows received 0.5 kg of a standard concentrate (887 g DM per kg; estimated NE: 7.7 MJ per
155 kg DM) when they were milked (i.e. 1.0 kg per day).

156 *2.3 Measurements and data analysis*

157 *2.3.1 Feeding behaviour*

158 For each visit to a feeder, RIC feeders recorded cow identity, the start time and end time (hh:mm:ss)
159 of the visit, and the start weight and end weight of the feed in the feeder to the nearest 0.1 kg. Visit
160 duration (s), feed intake (kg), and feeding rate (kg per min feeding) were calculated from these
161 records. Concentrate feeders only registered the amount of concentrate collected per cow per day.
162 RIC data were analysed from 6 weeks before calving until 7 weeks after calving. In total, 9 cows were
163 excluded from the analysis for various reasons: 5 cows were removed from the experiment before 7
164 weeks in lactation for health reasons (severe clinical lameness (2x), broken hip, 2 deaths within 10
165 days after calving), and 4 cows did not have the assigned DP length due to early calving in case of the
166 short DP group (n=1) and spontaneous drying off (i.e. the cow stopped lactating despite twice-daily
167 milking; n=3) in case of the no DP group. The RIC dataset consisted, therefore, of 122 13-week
168 periods, with a total of 332,524 recorded visits to the RIC feeders.

169 Criteria were used to clean the dataset prior to analysis. Visits with a feeding rate > 2 kg per min were
170 discarded (0.4% of records), because inspection of sequentially recorded visits to the same feeder
171 suggested that these records were erroneous. In addition, visit duration was discarded for visits that
172 lasted longer than 3 h and visits with feeding rates below 0.02 kg per min (0.1% of records).
173 Inspection of these records suggested that the recorded feed intakes were genuine for these visits, as

174 evidenced by sequentially recorded feeding bin weights, whereas visit durations were likely long
175 because of failed registration of the end time of the visit.

176 Visits were clustered based on the interval length between visits. For dairy cows, the distribution of
177 short intervals within meals and longer intervals between meals can be described by a three-
178 population model, which uses a combination of two Gaussian distributions for the short intervals and
179 one Weibull distribution for the longer intervals (for further details see: Yeates et al., 2001). A meal
180 criterion can be estimated from this distribution, to classify intervals as within meal and between meal
181 intervals in the most accurate way. When the interval between visits is shorter than the meal criterion,
182 the visits belong to the same meal.

183 Visit records were used to compute intervals between subsequent visits for each cow. A three-
184 population model was fitted to the frequency distribution of the \log_e -transformed intervals between
185 visits, and a meal criterion was estimated from this distribution (Yeates et al., 2001; Tolcamp et al.,
186 2002, 2011). To assess whether separate meal criteria for treatment groups or periods relative to
187 calving would be more appropriate than one single meal criterion for all treatments, nested models
188 were constructed. Three nested models were produced to estimate separate meal criteria for 1) the
189 three treatment groups (Short DP STD, No DP STD, and No DP LOW), 2) the two periods (before
190 and after calving), and 3) each treatment \times period interaction. Comparisons of the log-likelihoods of
191 nested models using likelihood ratio tests showed that the separate factors and their interaction all
192 improved model fit. However, the resulting meal criteria were very similar between treatment groups
193 before calving (18.1, 17.4, and 17.7 min) and after calving (21.9, 20.2, and 21.2 min). Therefore, it
194 was decided to use one meal criterion before calving (18.0 min) and one meal criterion after calving
195 (20.9 min), calculated from the pooled data. These meal criteria were used to cluster visits into meals.

196 Duration of meals (meal duration), duration of visits within meals (feeding duration), number of visits
197 per meal, and feed intake per meal were calculated, and secondary variables (e.g. daily feed intake,
198 feed duration and feeding rate) were derived from these variables. Weekly means of feeding
199 behaviour characteristics per cow per day were used for the analysis.

200 Mixed models were used to analyse the effect of fixed factors treatment, parity (1 or > 1 before
201 calving), and week, as well as interactions of parity and week with treatment, on feeding behaviour

202 (PROC MIXED procedure in SAS version 9.1; SAS Institute Inc., Cary, NC). The combination of
203 cow identity and parity before calving was specified as repeated subject. No DP STD and No DP
204 LOW were grouped together (No DP), because preliminary analysis showed no difference for this.
205 The covariance structure with the best model fit, based on the lowest Akaike's information criterion,
206 was selected from unstructured, compound symmetry, and autoregressive covariance structures.
207 Statistical significance ($P < 0.05$) of fixed effects was evaluated with approximate F tests (Kenward
208 and Roger, 1997); treatment contrasts were compared using Wald tests.

209 *2.3.2 Lying behaviour and steps*

210 Lying behaviour and steps were recorded with triaxial accelerometers (IceQube, IceRobotics, South
211 Queensferry, UK) from June 2014 until July 2015. Lying behaviour is recorded when the hind leg is
212 in a horizontal position; the step count measures the number of times the animal lifts its leg up and
213 places it back down again. The step count was used as indicator for walking activity, although
214 stepping may also be recorded while standing in one place (e.g. during milking). Sensors were
215 attached to the left or right hind leg and detached on Thursdays between 10.00 h and 12.00 h. Each
216 cow was herded into a cubicle for the attachment of the sensor. Because of limited sensor availability,
217 lying behaviour and steps of cows were recorded for 6 complete days (Friday until Wednesday) at 4
218 weeks (26 ± 3 to 21 ± 3 days) before the expected calving date, and at 4 weeks (22 ± 3 to 27 ± 3 days)
219 after calving only. Cows were regrouped and switched to a DP ration 11 days before the precalving
220 measurement period, and dried off 4 days before the precalving measurement period. We therefore
221 expect to measure little short-term behavioural responses to the change in diet, change in social
222 environment, or the process of drying off (von Keyserlingk et al., 2008; Tucker et al., 2009). Lying
223 behaviour and steps of 81 unique cows were recorded in both periods ($n=26$ for no DP STD; $n= 27$ for
224 no DP LOW; and $n= 28$ for short DP STD), including only cows that were also included in the
225 analysis of feeding behaviour.

226 Data were downloaded from IceQube sensors using IceReader, and processed by IceManager (both
227 from IceRobotics, South Queensferry, UK) to produce two data files per cow per time period. One file
228 contained all recorded lying bouts, with a start date, start time (hh:mm:ss) and duration (s); the other
229 file consisted of recorded lying time (s), standing time (s), and number of steps per 15-min interval.

230 Recorded lying bouts with durations shorter than 33 s were discarded as false lying bouts (Kok et al.,
231 2015). The filtered data of lying bouts were used to compute the number of lying bouts per cow per
232 day. Daily lying time and number of steps were computed from the 15-min summaries. Weekly means
233 of lying bouts, lying time and steps per cow per day were used for the analysis.

234 To analyse the effect of treatment (no DP or short DP), parity (1 or >1 before calving), and week on
235 lying time, number of lying bouts, and steps, the same mixed model approach was used as for the
236 analysis of feeding behaviour characteristics. No DP STD and No DP LOW were grouped together
237 (No DP), because preliminary analysis showed no difference for this.

238 The daily number of steps in the period before calving was compared between days of the week, to
239 assess the impact of going through the milking parlour for weighing on Tuesdays for cows with a
240 short DP. Per treatment, the mixed model included a fixed effect for day of the week (Friday through
241 Wednesday), and a random cow effect. All weekdays were compared using pairwise Wald tests with
242 Tukey-adjusted P-values, and the estimate statement was used to compare the number of steps
243 recorded on Tuesdays with all other days.

244 **2.3.3 Associations between behaviour, milk yield, and energy balance**

245 To assess possible reasons for differences in behaviour, we analysed associations between behaviour,
246 milk yield and energy balance at 4 weeks after calving. Milk yield was recorded daily. Energy balance
247 was calculated according to the Dutch net energy for lactation (VEM) system (Van Es, 1975) as the
248 difference between intake of VEM with the requirement of VEM for maintenance, milk production,
249 and pregnancy (1,000 VEM = 6.9 MJ of NE). Energy balance was expressed in $\text{kJ per kg}^{0.75}$ per day
250 (Van Es, 1975). Computation of the energy balance required milk yield, milk composition, body
251 weight of the cow, feed intake, and energy content of the feed. Milk samples for fat and protein
252 analysis (ISO 9622, Qlip, Zutphen, the Netherlands) were collected for four subsequent milkings and
253 were analysed as a weighted pooled sample per cow. RIC feeders recorded feed intake of the basal
254 ration (kg) and concentrate feeders recorded the quantity of concentrates dispensed (kg) per cow per d.
255 Feed intake was converted to energy intake using the dry matter content and net energy (NE) of each
256 diet component.

257 Means and standard deviations of milk yield and energy balance in week 4 after calving were

258 computed per DP treatment (no DP or short DP) per parity (2 or >2) . Associations between variables
259 were assessed with Pearson correlations. Significant correlations (r ; $P < 0.05$) were interpreted as
260 slight (< 0.2), low (0.2 - 0.4), moderate (0.4 - 0.7), high (0.7 - 0.9), or very high (> 0.9) (Martin and
261 Bateson, 1993).

262

263 **3. RESULTS**

264 *3.1 The effect of a short or no DP on feeding behaviour*

265 Over the 6 weeks before calving, cows with a short DP and cows with no DP had on average 7 meals
266 per day with 5 visits per meal from the RIC feeders (i.e. excluding concentrate; Table 1). Average
267 meal duration (i.e. the time from the start of the first visit within the meal until the end of the last visit
268 within the meal), however, was longer for cows with a short DP than for cows with no DP, which
269 resulted in total meal times of 293 min per day for cows with a short DP and 255 min per day for
270 cows with no DP. The feeding duration (i.e. the time spent with head in the feeder) was about 80% of
271 the meal duration for both treatments. Meal size (i.e. the feed intake per meal) and feed intake per day
272 were smaller for cows with a short DP than for cows with no DP, and cows with a short DP had a
273 lower feeding rate. Young cows (parity 1) had longer total feeding times and lower feeding rates than
274 older cows (parity >1) (Figure 1c, 1d).

275 Over the 7 weeks after calving, cows with a short DP and cows with no DP had on average 8 meals
276 per day with 4 visits per meal from the RIC feeders (Table 2). The number of meals per day and
277 feeding rate peaked in the first week after calving, whereas total feeding time and feed intake were
278 lowest in this week (Figure 1). Average meal duration and total meal time were not different between
279 DP treatments after calving. Meal size was not different between cows with a short DP and cows with
280 no DP, but feed intake per day was 3.4 kg (1.3 kg DM) per day lower for cows with a short DP than
281 for cows with no DP. Young cows (parity 1 before calving) with a short DP had longer total feeding
282 times ($P = 0.04$) and lower feeding rates ($P < 0.01$) than young cows with no DP (Figure 1c, 1d).

283 Looking at the diurnal pattern of feeding, cows spent more time in a meal during daytime than during
284 the night, with the highest proportion of cows having meals after fresh feed delivery, peaking around
285 noon (Figure 2). Before calving, cows with a short DP spent more time in a meal during daytime than
286 cows with no DP (Figure 2a). After calving, the diurnal pattern of meals was similar for cows with a
287 short DP and cows with no DP (Figure 2b).

288

289 ***3.2 The effect of a short or no DP on lying behaviour and steps***

290 The number of lying bouts per day was not affected by DP treatment or period relative to calving
291 (Table 3). Young cows (parity 1 before calving) had 13.2 (SE: 0.5) lying bouts per day, whereas older
292 cows (parity > 1) had 11.4 (SE: 0.5) lying bouts per day ($P < 0.01$).

293 Daily lying time was affected by a DP treatment \times period interaction. At 4 weeks before calving, daily
294 lying time was 1.1 h longer for cows with a short DP than for cows with no DP ($P = 0.01$). At 4 weeks
295 after calving, however, daily lying time was 0.9 h shorter for cows with a short DP than for cows with
296 no DP ($P = 0.03$). The change in lying time between the period before calving and the period after
297 calving was more extreme for cows with a short DP (-3 h) than for cows with no DP (-1 h).

298 Before calving, time spent lying dipped during milking for cows with no DP, whereas this was not the
299 case for cows with a short DP (Figure 3a). After calving, lying patterns were similar for cows with a
300 short DP and cows with no DP (Figure 3b).

301 The number of steps per day was affected by a DP treatment \times period interaction (Table 3). Before
302 calving, cows with a short DP had 41% lower step counts than cows with no DP. After calving, the
303 number of steps did not differ between DP treatments, and was similar to the number of steps of cows
304 with no DP during the period before calving.

305 Before calving, cows with a short DP were weighed in the milking parlour on Tuesdays. To assess the
306 impact of this additional exercise on daily number of steps, step counts were compared between days
307 of the week. On average, 220 (SE: 29) more steps were recorded for cows with a short DP on
308 Tuesdays than on Wednesdays through Mondays ($P < 0.01$; Figure 4). Cows with a short DP also had

309 lower step counts during weekends than on weekdays. Cows with no DP had no increased step count
310 on Tuesdays during the period before calving. Their step count was highest on Mondays, which was
311 the day animals were regrouped (although focal cows were not moved in this period).

312 *3.3 Associations between behaviour, milk yield, and energy balance.*

313 Mean milk yield of cows with a short DP was 37.9 kg (SD: 6.0) per day for cows in parity 2 and 36.8
314 kg (SD: 6.3) per day for older cows; mean milk yield of cows with no DP was 29.5 kg (SD: 5) per day
315 for cows in parity 2 and 35.3 kg (SD: 8.1) per day for older cows. Mean energy balance of cows with
316 a short DP was -191 kJ per kg^{0.75} (SD: 150) per day for cows in parity 2 and -179 kJ per kg^{0.75} (SD:
317 190) per day for older cows; mean energy balance of cows with no DP was 44 kJ per kg^{0.75} (SD: 113)
318 per day for cows in parity 2 and -94 kJ per kg^{0.75} (SD: 193) per day for older cows.

319 Dry matter intake and basal ration intake were lowly positively correlated with milk yield (Table 4).

320 No correlations with milk yield were found for other variables of feeding behaviour (feeding rate,
321 feeding and meal duration, and number of visits and meals). A low negative correlation was found
322 between daily lying time and milk yield. Steps and number of lying bouts were not correlated with
323 milk yield.

324 Low positive correlations were found between energy balance and number of meals, and between
325 energy balance and number of visits. Feeding rate and total feeding time were not correlated with
326 energy balance, but were highly negatively correlated with each other ($r: -0.71; P < 0.01$).

327 Low positive correlations were found between daily lying time and energy balance, dry matter intake
328 ($r: 0.26; P: 0.02$), and basal ration intake ($r: 0.32, P < 0.01$). There were no correlations, however,
329 between total feeding time or total meal time and daily lying time. Number of steps had low positive
330 correlations with energy balance, daily meal duration ($r: 0.38; P < 0.01$), and daily feeding duration ($r:$
331 $0.29; P: 0.01$); and a moderate correlation with the number of visits to the feeder ($r: 0.54; P < 0.01$).

332

333 **4. DISCUSSION**

334 Compared with cows with no DP, cows with a short DP spent a longer time feeding but had a reduced
335 feed intake (kg and NE) before calving. Other experimental studies with different DP lengths also

336 reported lower feed intake (DM) before calving for cows with a conventional or short DP than for
337 cows with no DP (Rastani et al., 2005; van Knegsel et al., 2014). In the current study, the DP was
338 accompanied by a DP ration, as is common in commercial dairy farming (Rastani et al., 2005;
339 Santschi et al., 2011; Steeneveld et al., 2013). It is unlikely, however, that the reduced feed intake was
340 due to the change from the lactation ration to the DP ration, because this reduction was also observed
341 for cows with a short DP without a ration change (Rastani et al., 2005). This lower feed intake of dry
342 cows likely reflects the lower energy requirement of dry cows. The lower feeding rate during the DP
343 may be related to the high amount of fibre and the lower palatability of the DP ration (Baumont, 1996;
344 Friggens et al., 1998).

345 Calving and the associated management had a large impact on feeding behaviour, irrespective of DP
346 treatment. In the first week after calving, cows had more frequent, but shorter, meals, and increased
347 feeding rates, overall resulting in a lower feed intake than in subsequent weeks. This suggests that the
348 impact of calving and its associated management is not so much related to DP-related ration or group
349 changes, and that the periparturient period will remain a period with quite extreme behavioural
350 changes. Cows had about 7 meals per day before calving, and 8 meals per day after calving,
351 irrespective of DP treatment. The use of separate meal criteria for feeding behaviour before calving
352 and after calving did not influence this difference: conclusions were similar when a single meal
353 criterion was used for both periods.

354 After calving, feed intake remained lower for cows with a short DP than for cows with no DP, despite
355 being fed the same diet. Rastani et al. (2005) also reported a lower feed intake for cows with a short
356 DP than for cows with no DP in the first 3 weeks after calving, whereas Van Knegsel et al. (2014) did
357 not find a difference in feed intake between cows with a short DP and cows with no DP in the first 14
358 weeks after calving. It is unclear why cows with a short DP had a lower feeding rate than cows with
359 no DP after calving. This might be related to rumen adaptation after a change in diet (Martens et al.,
360 2012), the onset of lactation, or the change of social environment after calving. Further studies are
361 needed in order to disentangle the impact of these factors. Possibly, cows with a short DP experience
362 more inappetence or discomfort due to a more severe negative energy balance (Roche et al., 2009).

363 The higher energy intake and lower milk yield of cows with no DP compared with cows with a short
364 DP may reduce the risk of metabolic diseases and improve welfare in early lactation (Ingvarlsen,
365 2006).

366 Cows with a short DP on average had a lower step count before calving than cows with no DP. This
367 could be a direct consequence of the absence of the milking procedure, because going through the
368 milking parlour (to be weighed) increased the step count of cows with a short DP by 220 steps.
369 Excluding the day of weighing, cows with a short DP performed on average 624 steps per day, and
370 cows with no DP performed 1117 steps per day. Twice-daily milking, therefore, could explain 89% of
371 the difference $((2 \times 220)/(1117 - 624))$ in step count between cows with a short DP and cows with no
372 DP, which suggests that the difference in steps was due to a difference in walking distance. Stepping
373 could also occur without walking, e.g. as a restless behaviour in the milking parlour (Gygax et al.,
374 2008). However, with stepping rates of less than 1 step per minute during the preparation and milking
375 phases (Gygax et al., 2008), this is unlikely to contribute much to the observed difference in steps per
376 day. Pen size was smaller for dry cows than for lactating cows, because the density was maintained at
377 7 m^2 per cow and the dry cow group mostly consisted of 6 or fewer cows at a time. This could have
378 further reduced the number of steps of cows with a short DP. Previous research showed that lactating
379 cows walked more in larger pens (Telezhenko et al., 2012).

380 It could be questioned whether the reduced number of steps during the DP is beneficial, or whether
381 the reduced physical activity might be a risk factor for cow health. Walking distance of housed cows
382 is already limited compared with walking distance of grazing cows. For example, studies reported
383 walking distances of 233 m per day for housed cows versus 2170 m for cows on pasture (Olmos et al.,
384 2009), and step counts of 1506 versus 4064 steps per day (Dohme-Meier et al., 2014). Studies showed
385 that exercise is beneficial for health in early lactation and for fitness of lactating and dry dairy cows
386 (Gustafson, 1993; Davidson and Beede, 2009). In humans, women who continued to exercise
387 regularly throughout pregnancy had a lower incidence of operative delivery, and had shorter active
388 labour than women who discontinued their exercise (Clapp, 1990).

389 Before calving, the daily lying time of cows with no DP (12.6 h) was lower than for cows with a short
390 DP (13.7 h), but higher than previously reported lying times of dry cows of 11.7 and 12.2 h per day
391 (Huzzey et al., 2005; Schirmann et al., 2011). These lying times likely reflect the overall response to
392 the environment in late gestation, as opposed to a short-term response to changes in management,
393 because cows were regrouped and rations were switched 11 days before the measurement period, and
394 cows were dried off 4 days before the measurement period. Cows in both DP treatments spent less
395 time lying after calving than before calving. Other studies found that lying time was lower in early
396 lactation than later in lactation (Munksgaard et al., 2005; Bewley et al., 2010). Due to the short lying
397 time, hormonal changes, and a negative energy balance, cows in early lactation are particularly
398 susceptible to lameness (Cook and Nordlund, 2009). The no DP treatment increased daily lying time
399 after calving by 0.9 h compared with a short DP, which might reduce the risk of developing lameness
400 in early lactation.

401 Higher daily lying time was lowly associated with lower milk yield in early lactation. It has been
402 suggested that cows with higher yields and a more severe negative energy balance have to spend more
403 time feeding, and can consequently spend less time lying (Roche et al., 2009; Bewley et al., 2010). In
404 the current study, however, cows with a short DP and cows with no DP spent less time feeding and
405 less time lying after calving than before calving. For cows with a short DP, the reduction in feeding
406 and lying time may be related to the twice-daily milking after calving, compared with no milking
407 before calving, that reduced their time budget for other behaviours. This was not the case for cows
408 with no DP, however, because they were milked both before and after calving. Moreover, daily
409 feeding time was not associated with daily lying time, or with milk yield. Therefore, lying time was
410 probably not constrained by feeding time. Norrington et al. (2012) also found a negative association
411 between daily lying time and milk yield at 8 weeks in milk, with no associations between milk yield
412 and feeding time. Løvendahl and Munksgaard (2016) found a positive correlation between milk yield
413 and feeding time and a negative correlation between milk yield and lying time in primiparous cows.
414 Other factors may explain why a higher milk yield was associated with a shorter lying time in early
415 lactation. Considering that level of milk yield relates to udder pressure (Bertulat et al., 2013), cows

416 with higher milk yields may experience discomfort when lying down and therefore lie down less.
417 There was a low positive association between energy balance and daily lying time in this study. A
418 prolonged negative energy balance might also cause discomfort due to hunger, weariness, or
419 (subclinical) metabolic disorders, which might reduce lying behaviour (Roche et al., 2009).
420 Not subjecting cows to a DP could improve cow welfare. It does not require cessation of lactation, or
421 ration and group changes commonly associated with a DP. During late gestation, cows with no DP
422 spent more than 12 h per day lying, without the reduction in steps that was seen in cows with a short
423 DP. In early lactation, cows with no DP had a higher feed intake, improved energy balance, and
424 increased lying time compared with cows with a short DP. In the current study, the impact of being
425 dry cannot be separated from the impact of group and ration changes. The impact of DP management
426 might be lessened through technical solutions. For example, separation gates can divert dry cows
427 away from the milking parlour and towards a DP ration, and thereby facilitate that dry cows remain in
428 the lactating herd. Moreover, a short DP can be applied without a change in ration (Rastani et al.,
429 2005). An experimental design in which dry and lactating cows remain in the same herd is necessary
430 to research the impact of being dry as such.

431 **5. CONCLUSION**

432 Cows with a short DP appeared to get more rest than cows with no DP: they had lower step counts
433 and longer lying and feeding times in late gestation. The differences in number of steps and feed
434 intake seemed direct consequences of not being milked. Cows with no DP also had longer lying times
435 (exceeding 12 h per day) before calving than in early lactation, despite the twice-daily milking. After
436 calving, cows with no DP had longer lying times and ate more than cows with a short DP. Not
437 subjecting cows to a DP may improve cow welfare through absence of DP-related changes in
438 management (i.e. cessation of lactation, ration and group changes), increased walking activity in late
439 gestation, and a better feed intake and longer lying time in early lactation.

440 **ACKNOWLEDGEMENT**

441 The authors thank the staff of the Dairy Campus and P. van der Spek for support during the
442 experiment, and C. G. van Reenen and J. T. N. van der Werf for sharing the IceManager software.

443 This study is financed by DairyNL (Zuivel NL; organization of the Dutch dairy supply chain) and the
444 Dutch Ministry of Economic Affairs (EZ), as part of the Sustainable Dairy Chain initiative (Duurzame
445 Zuivelketen). The authors gratefully acknowledge DairyCare (COST Action FA1308) for funding a
446 Short Term Scientific Mission for the first author at SRUC.

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